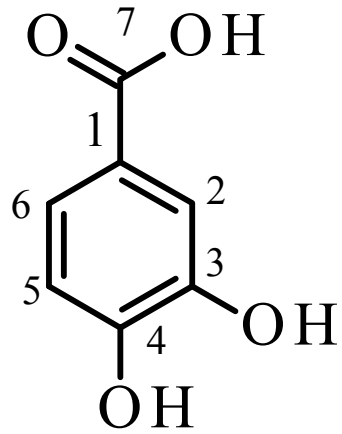
Supplementary Materials: On-Line Screening, Isolation and Identification of Antioxidant Compounds of *Helianthemum ruficomum*

Yasmine Chemam, Samir Benayache, Eric Marchioni, Minjie Zhao, Paul Mosset and Fadila Benayache

List of Figures

Molecule 1: Protocatechuic acid



1

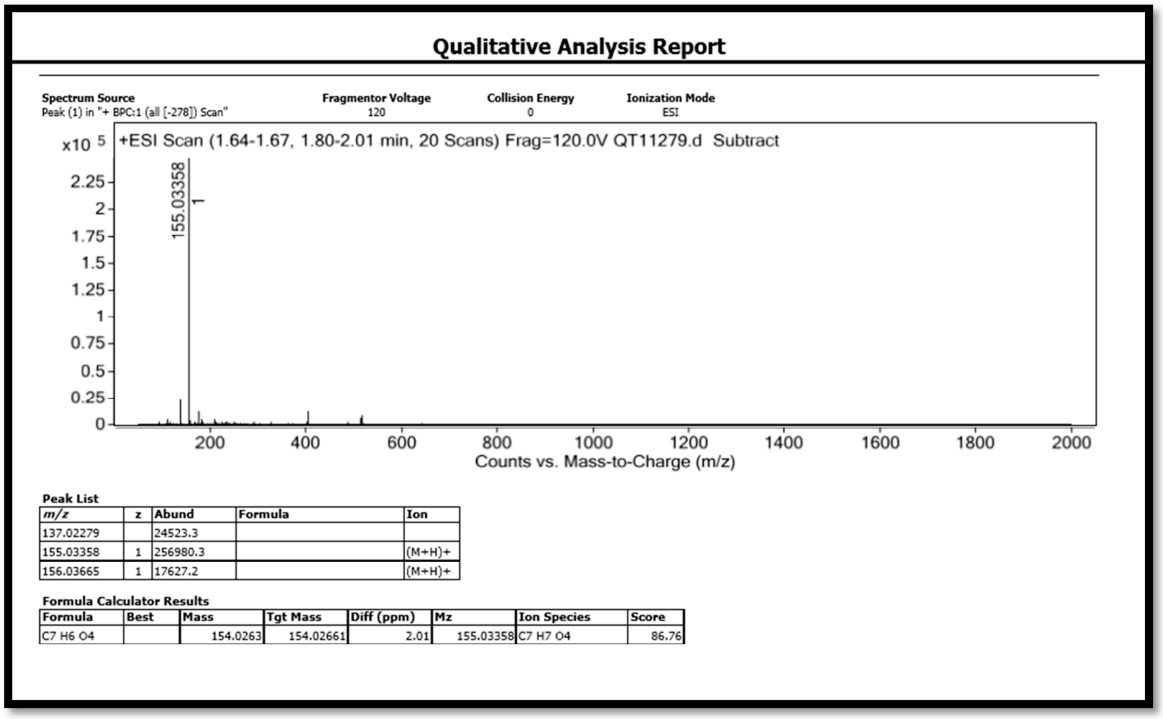


Figure S1. ESI-HRMS(+) of Protocatechuic acid.

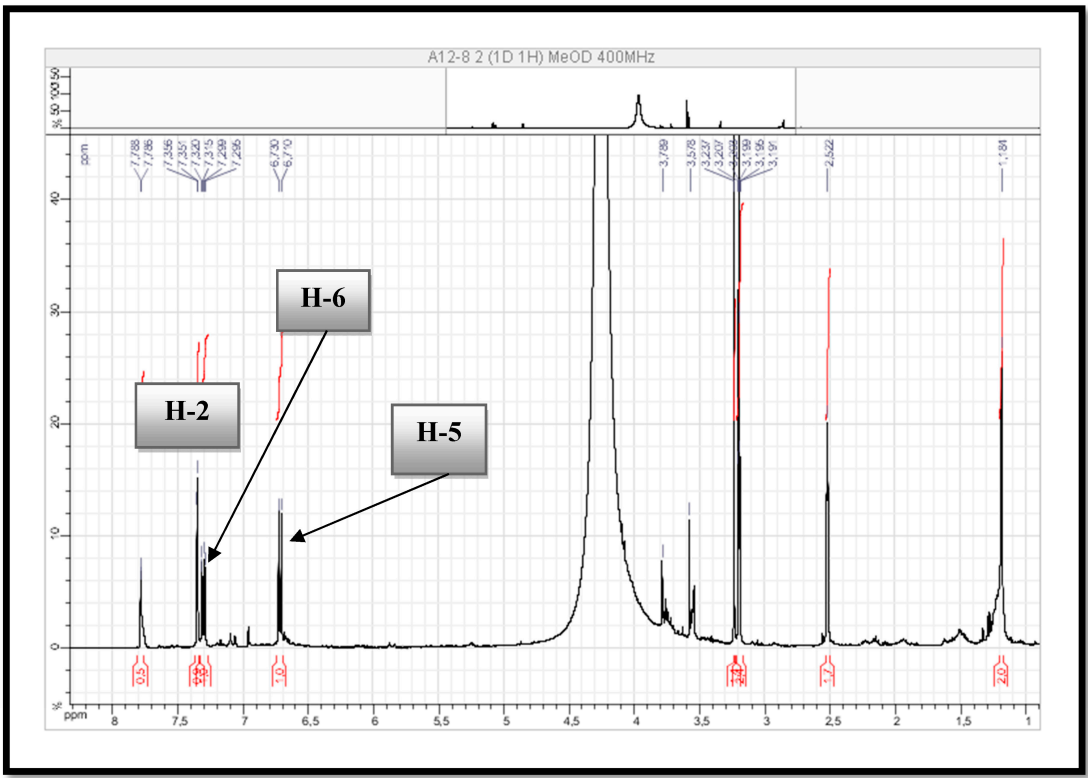


Figure S2. ^{1}H NMR spectrum (400 MHz, CD_3OD, δppm) of protocatechuic acid.

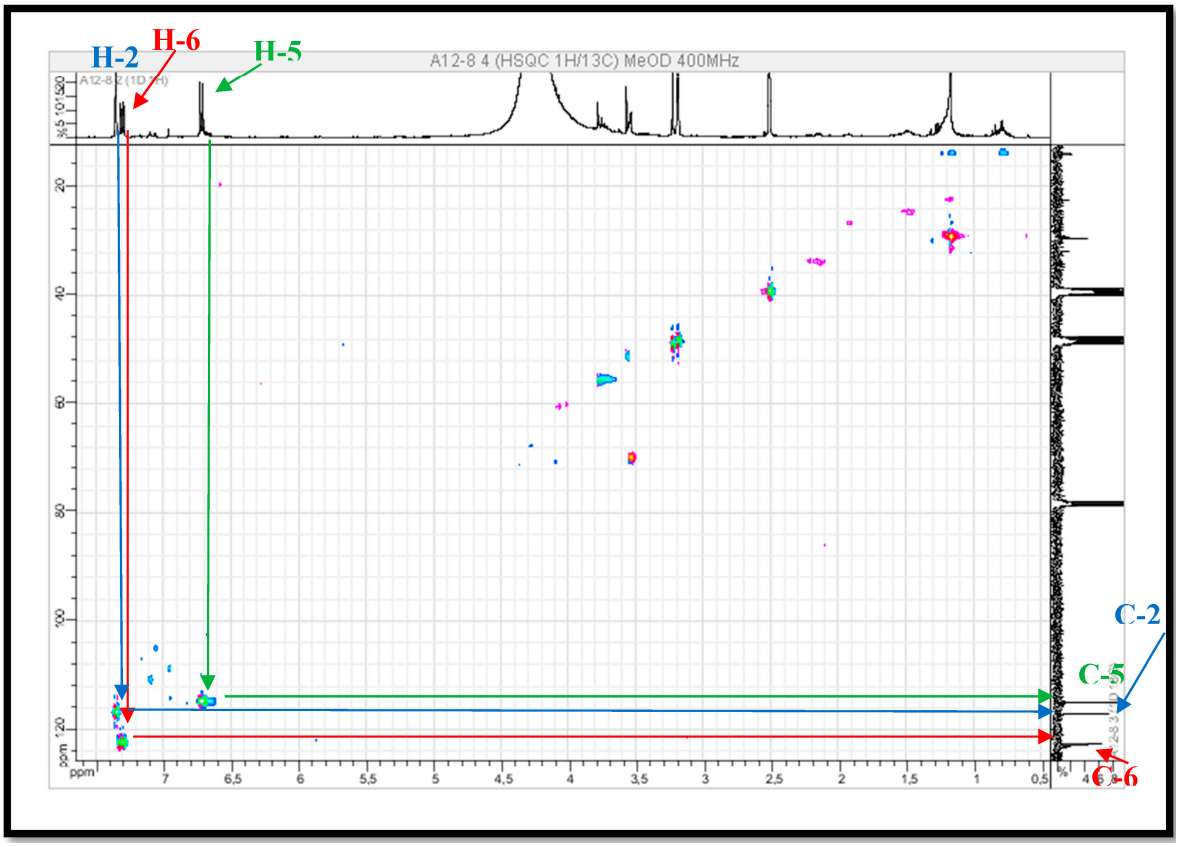


Figure S3. HSQC spectrum (400 MHz, CD_3OD, δppm) of protocatechuic acid.

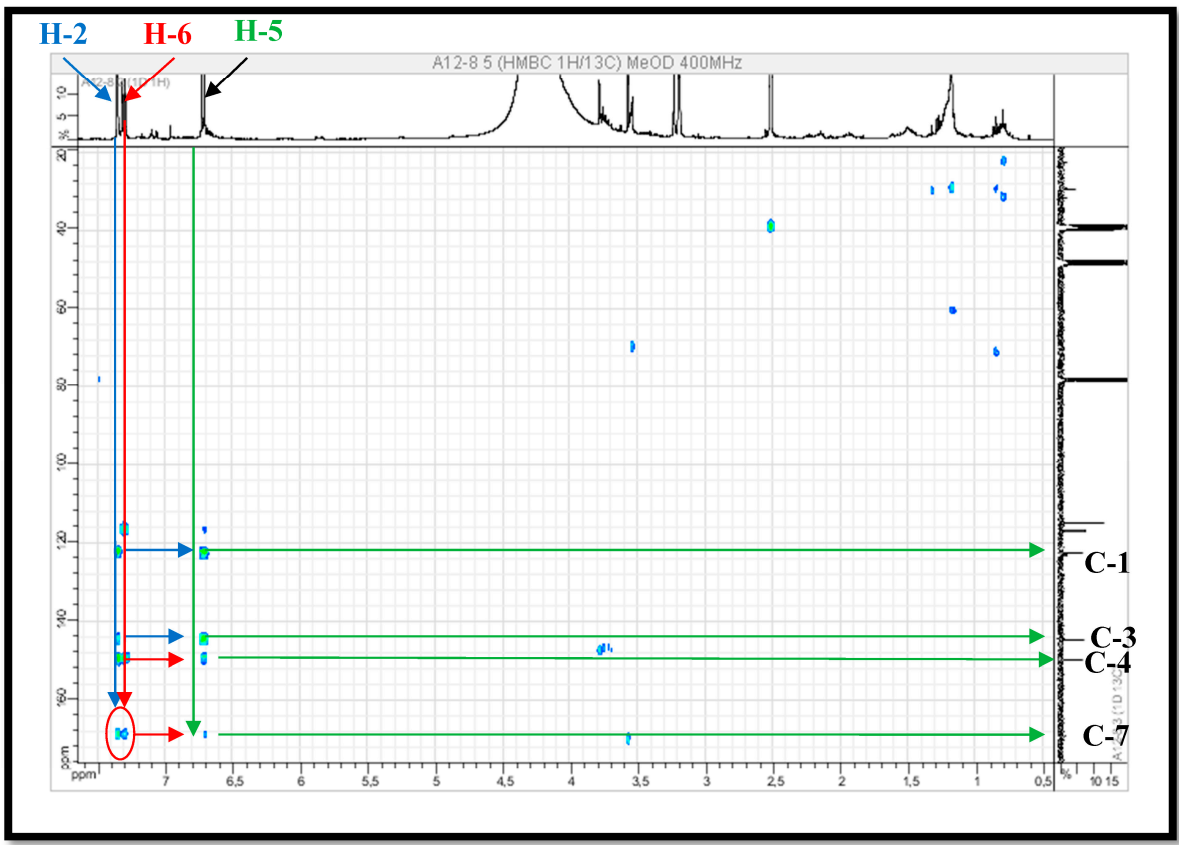


Figure S4. HMBC spectrum (400 MHz, CD_3OD, δppm) of protocatechuic acid.

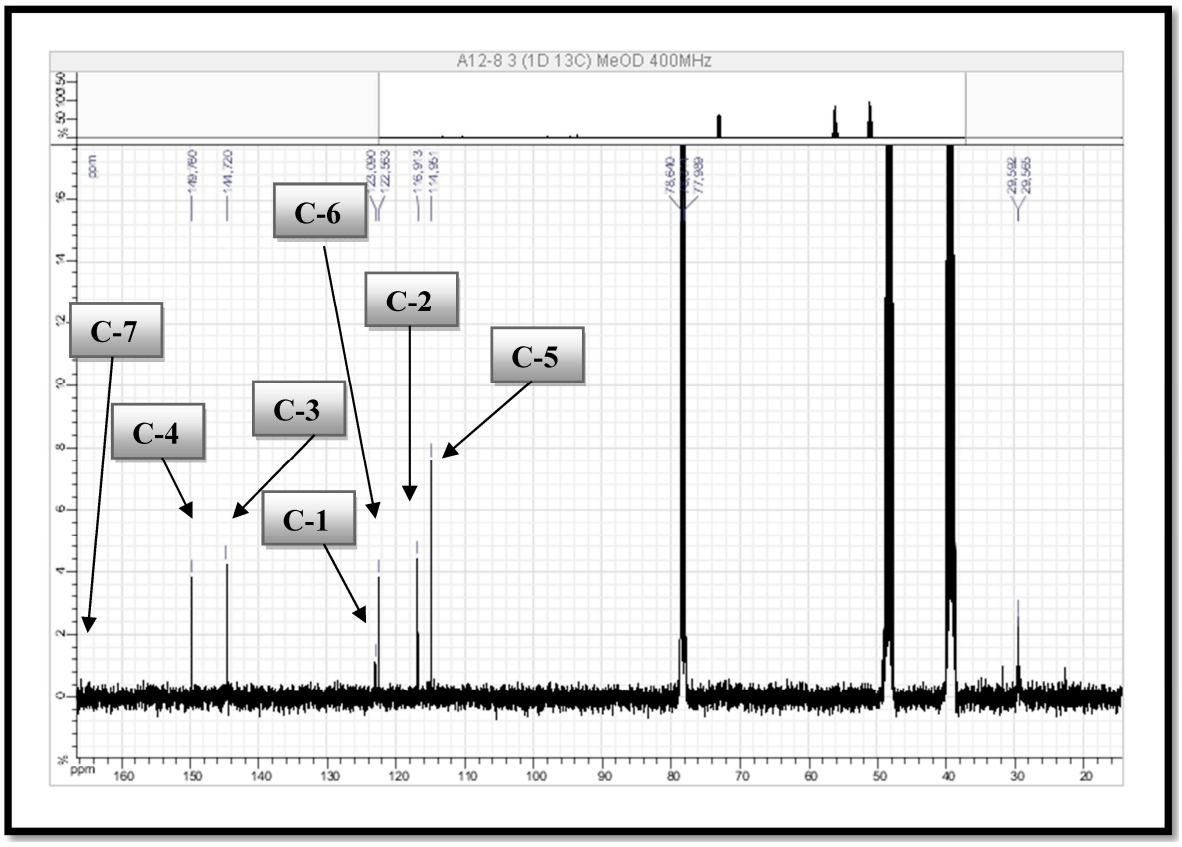
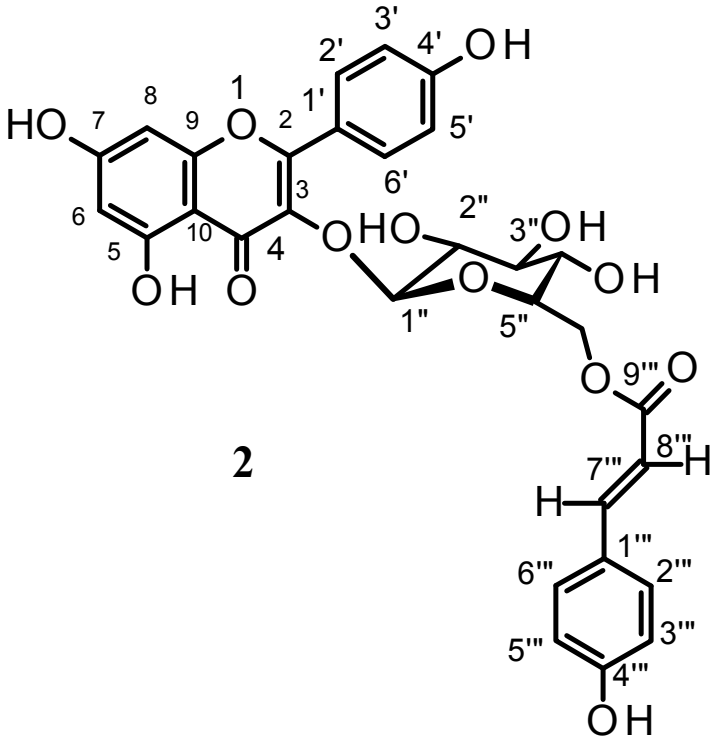


Figure S5. ^{13}C NMR spectrum (100 MHz, CD_3OD, δppm) of protocatechuic acid.

Molecule 2: *Trans*-tiliroside



2

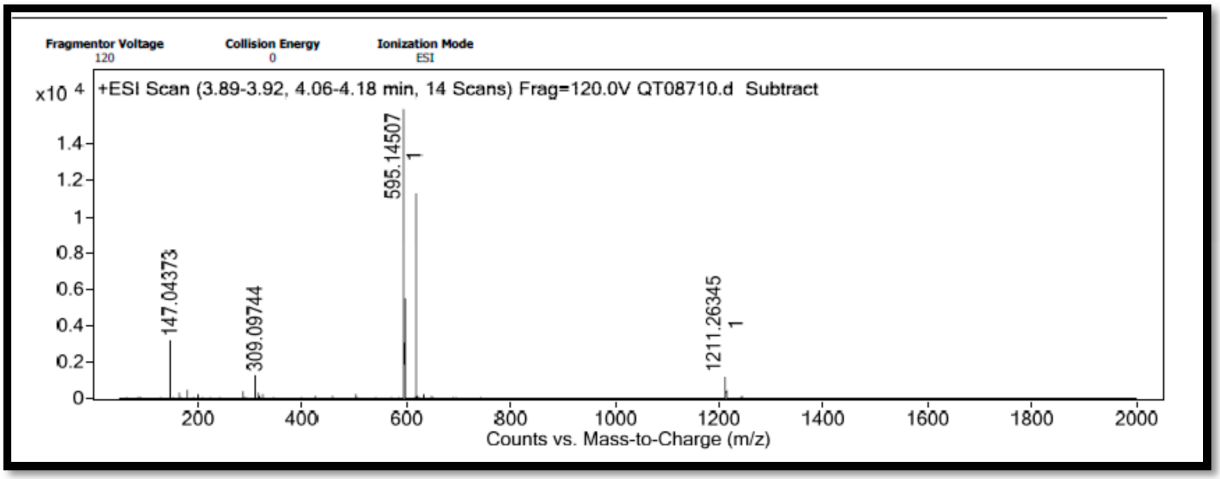


Figure S6. ESI-HRMS(+) of *trans*-tiliroside.

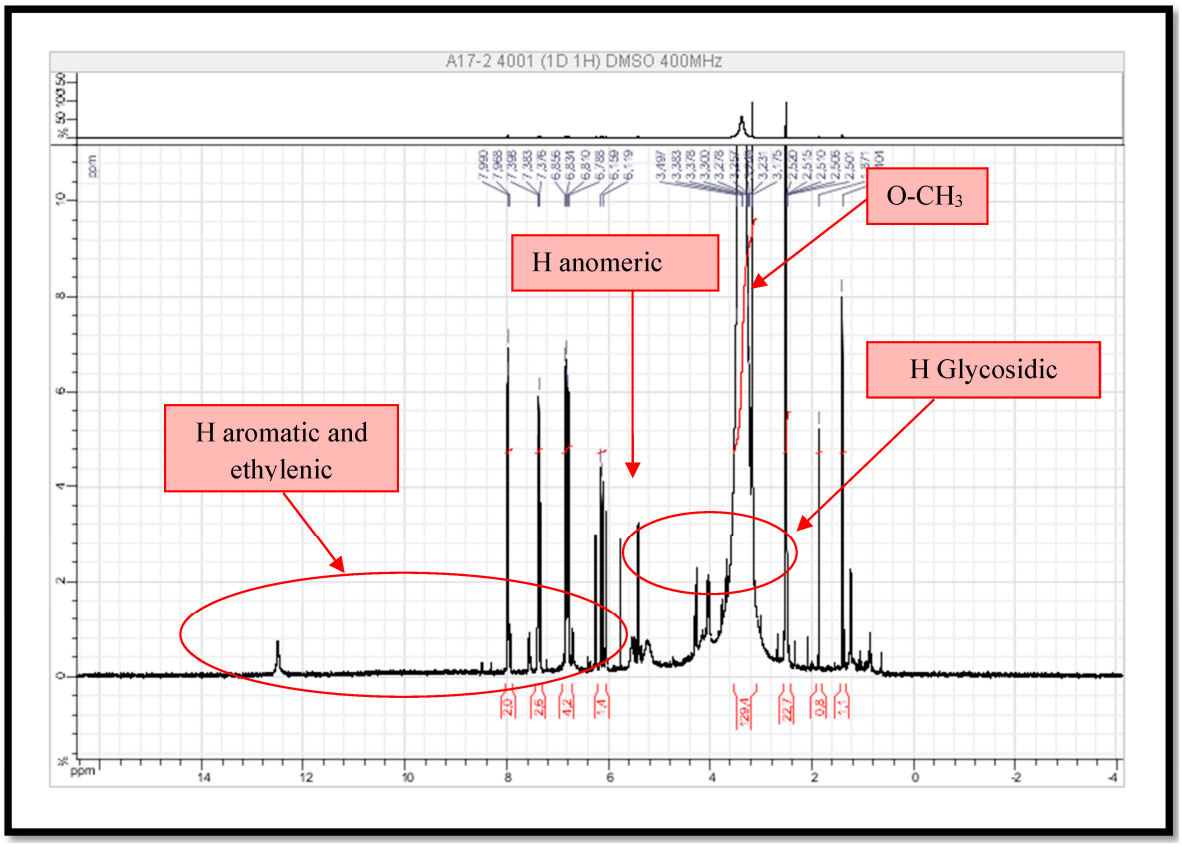


Figure S7. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

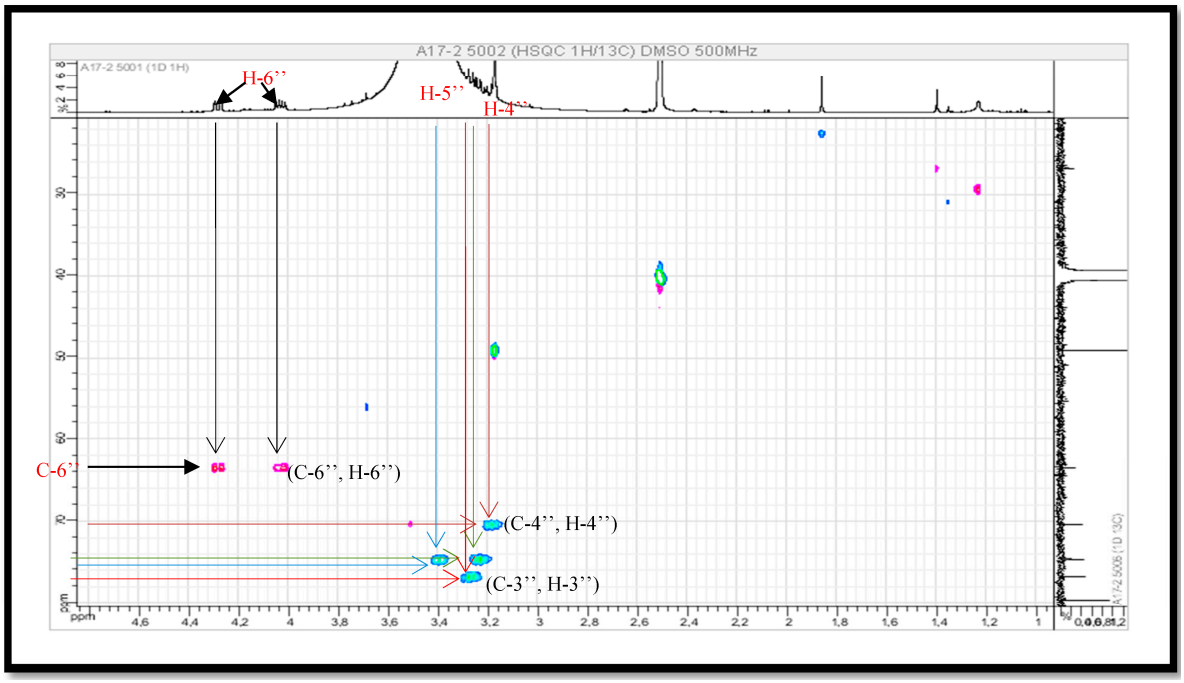


Figure S8. HSQC spectrum (spreading out 1) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

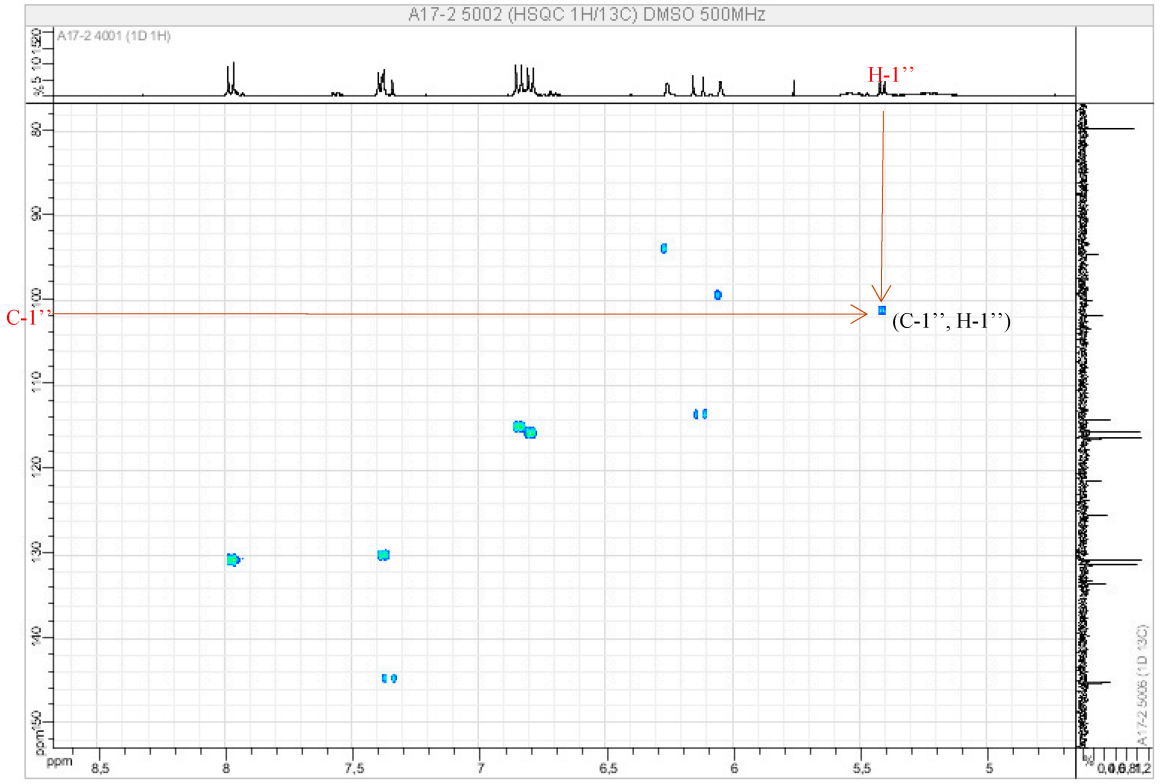


Figure S9. HSQC spectrum (spreading out 2) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

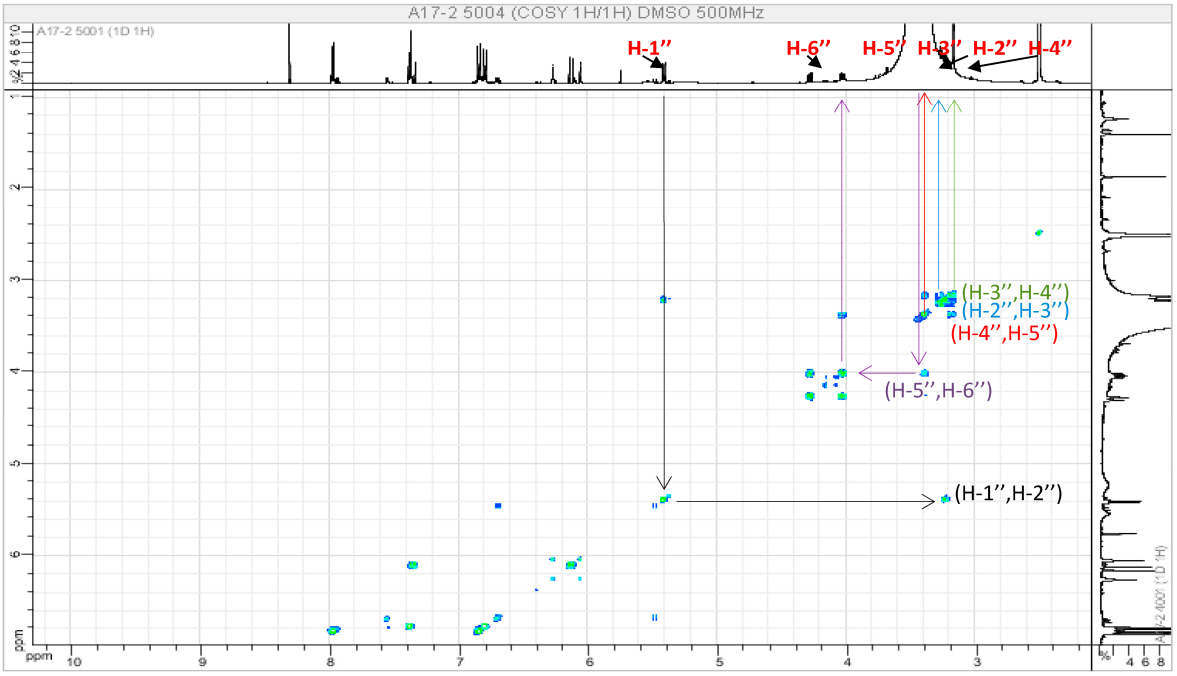


Figure S10. COSY spectrum (spreading out 1) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

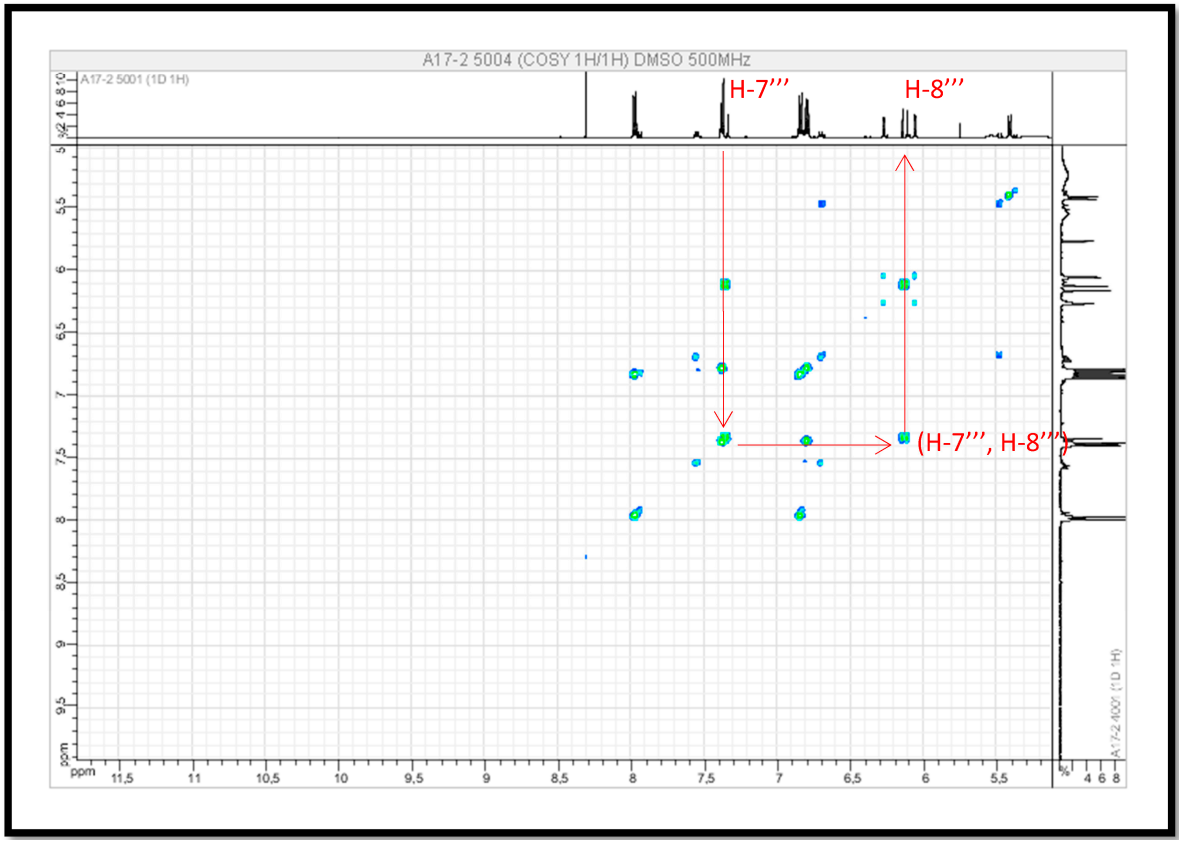


Figure S11. COSY spectrum (spreading out 2) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

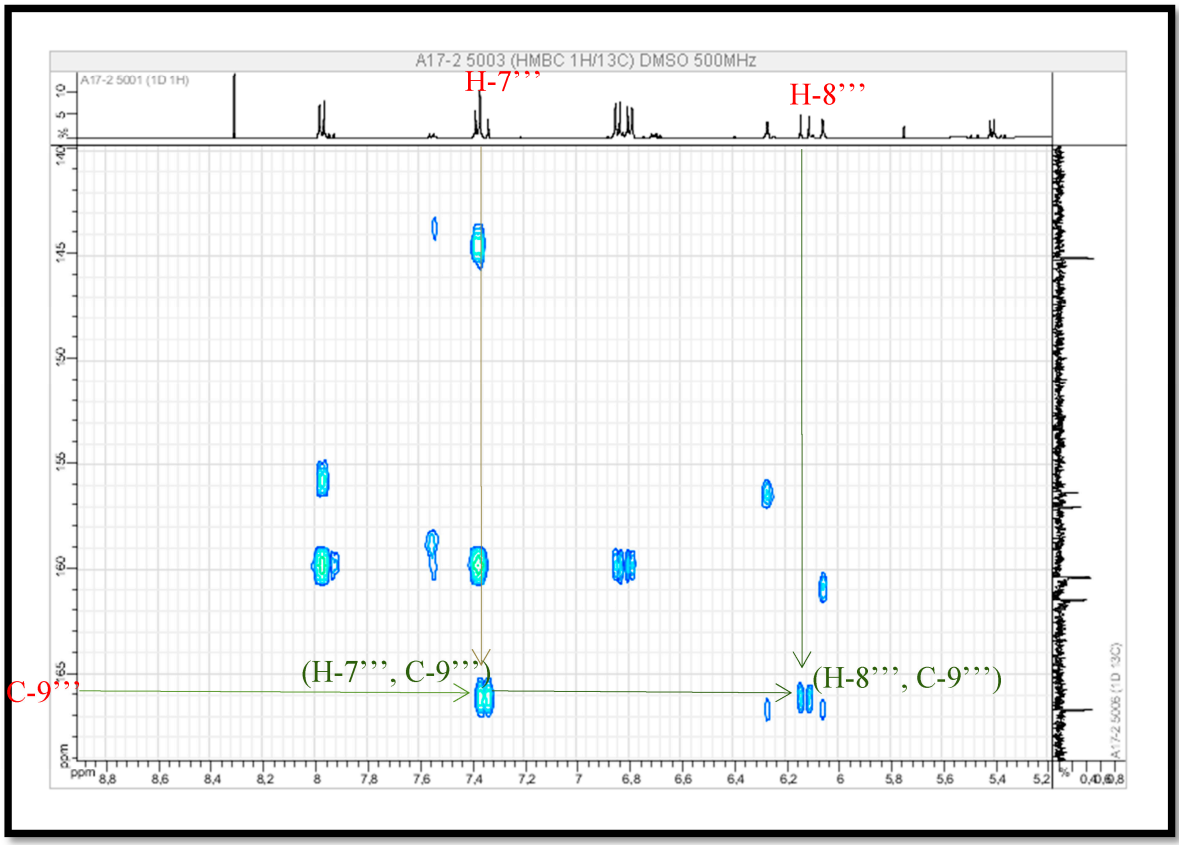


Figure S12. HMBC spectrum (spreading out 1) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

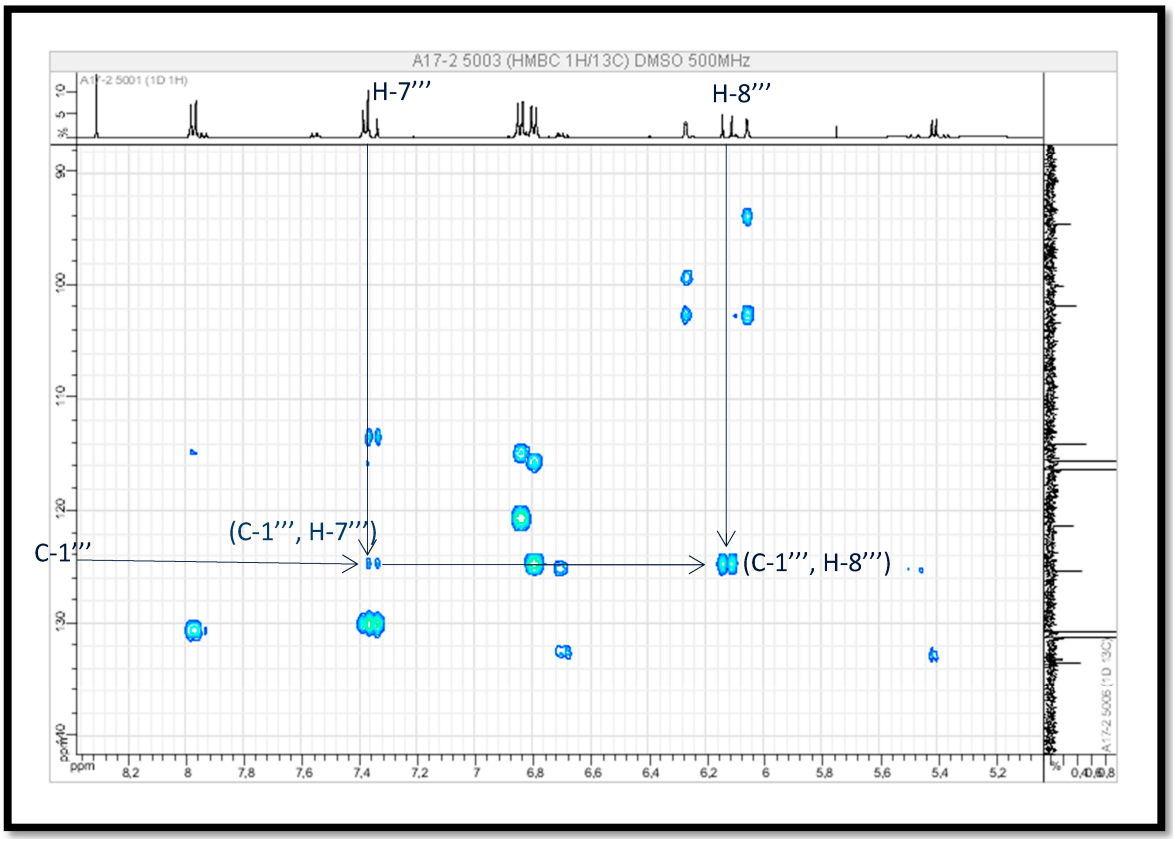


Figure S13. HMBC spectrum (spreading out 2) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

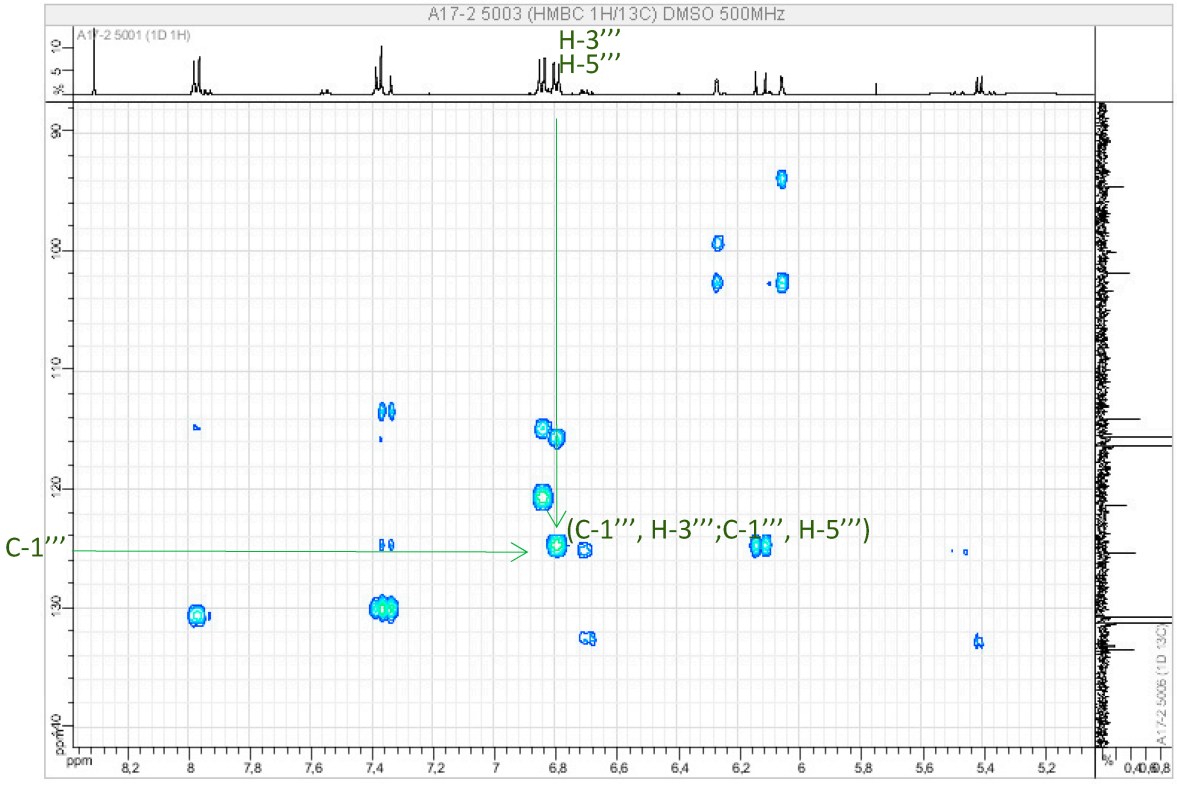


Figure S14. HMBC spectrum (spreading out 3) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

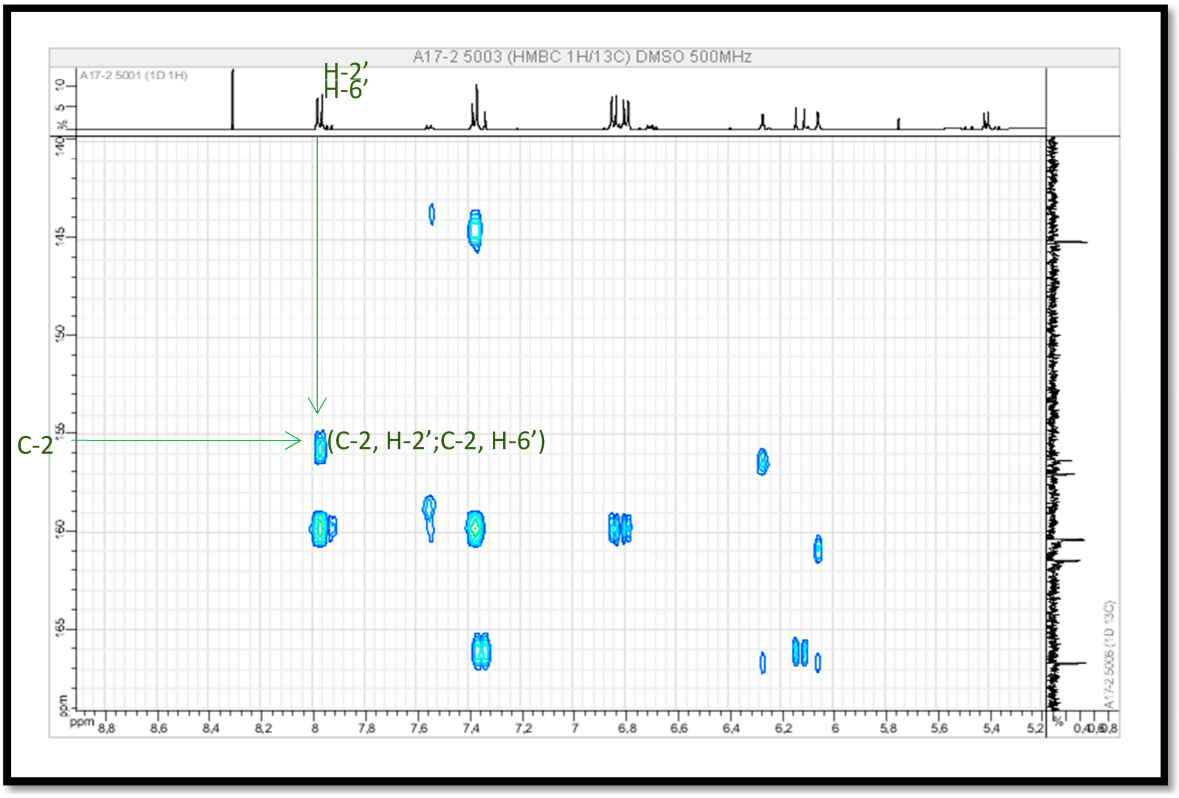


Figure S15. HMBC spectrum (spreading out 4) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

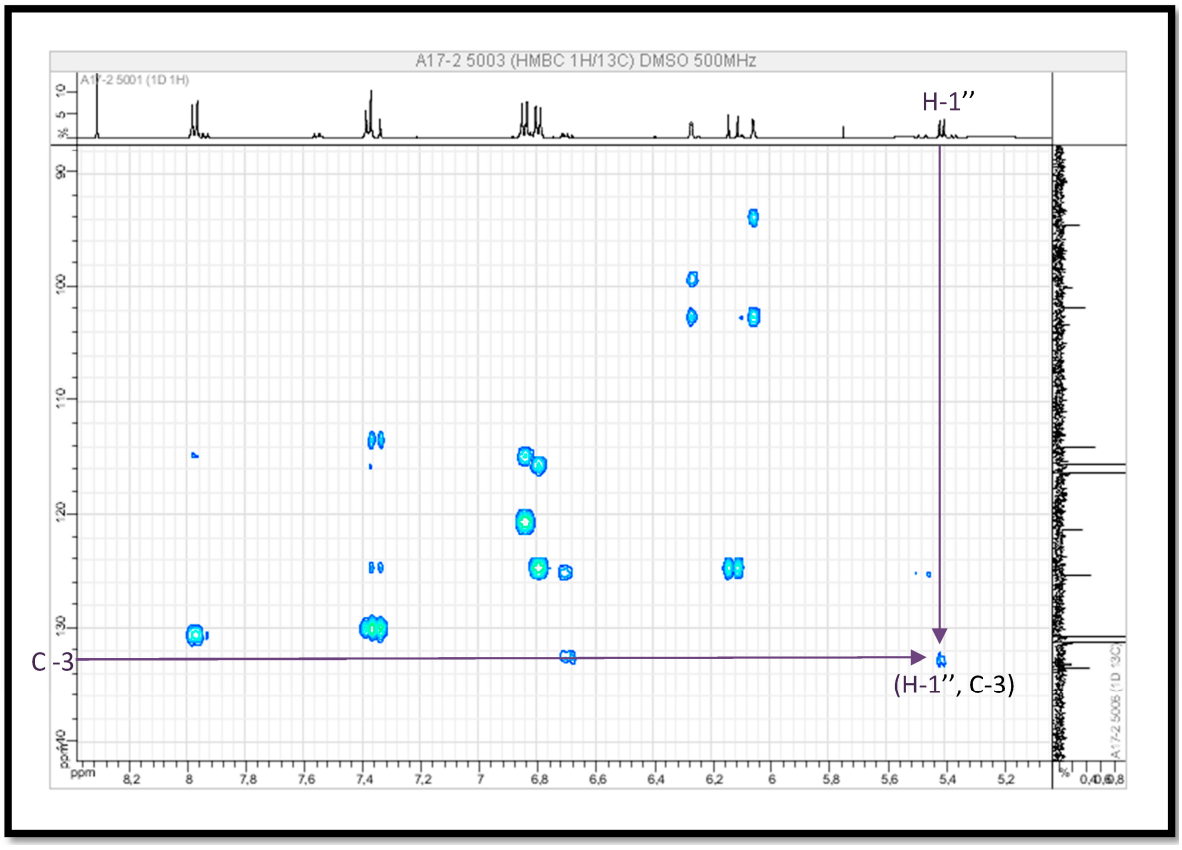


Figure S16. HMBC spectrum (spreading out 5) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

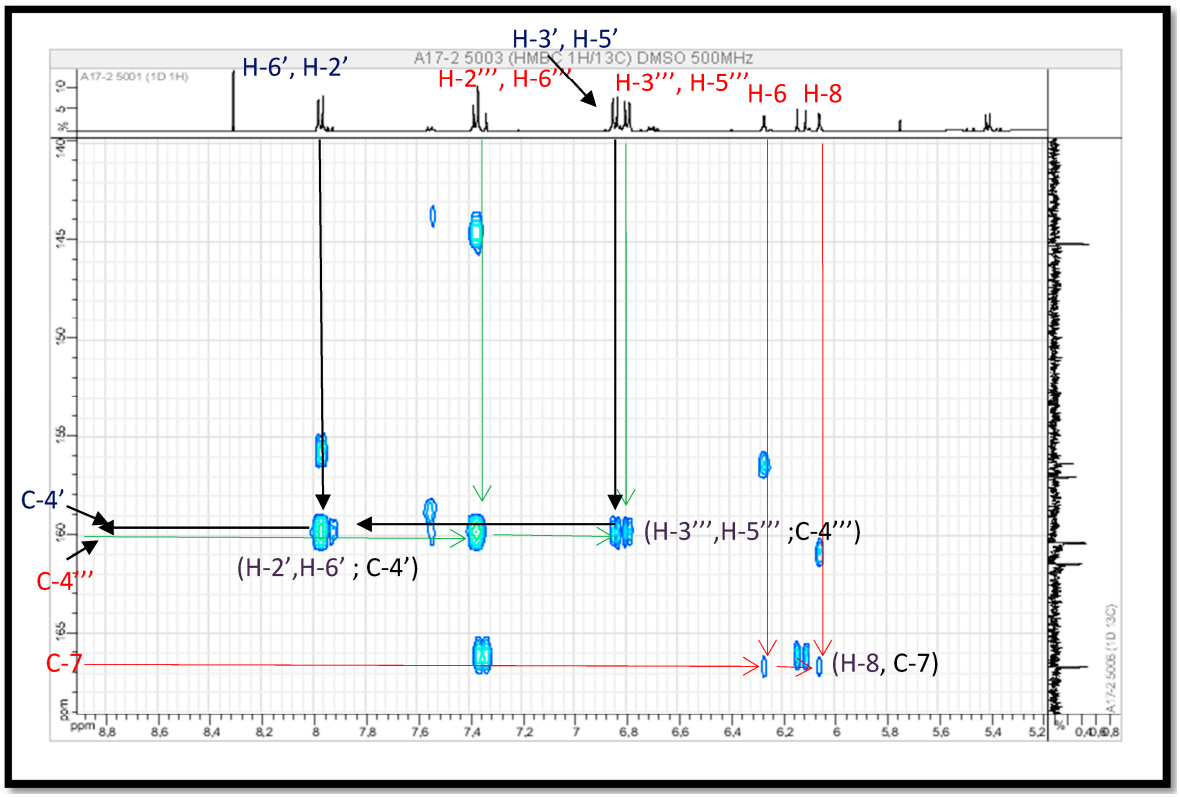


Figure S17. HMBC spectrum (spreading out 6) (500 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

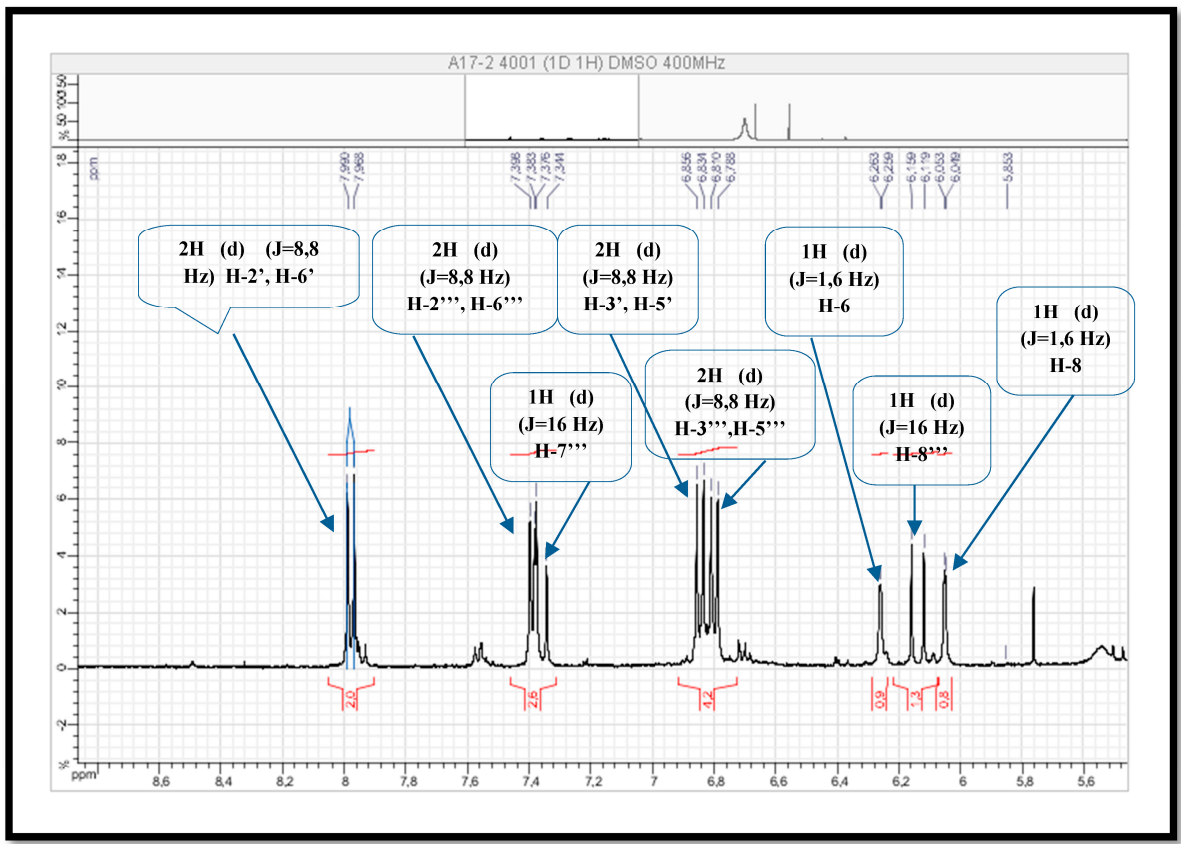


Figure S18. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

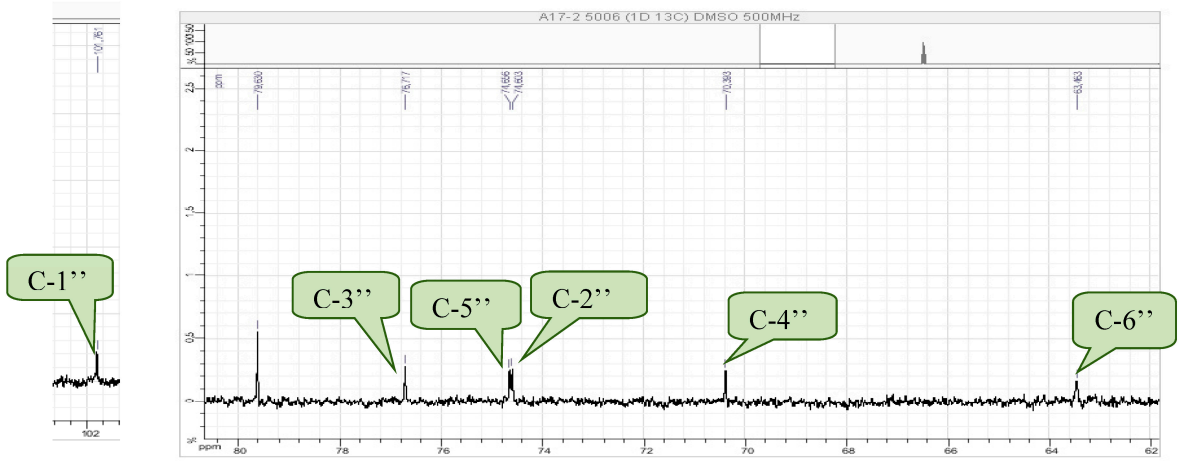


Figure S19. ^{13}C NMR spectrum (spreading out 1) (125 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

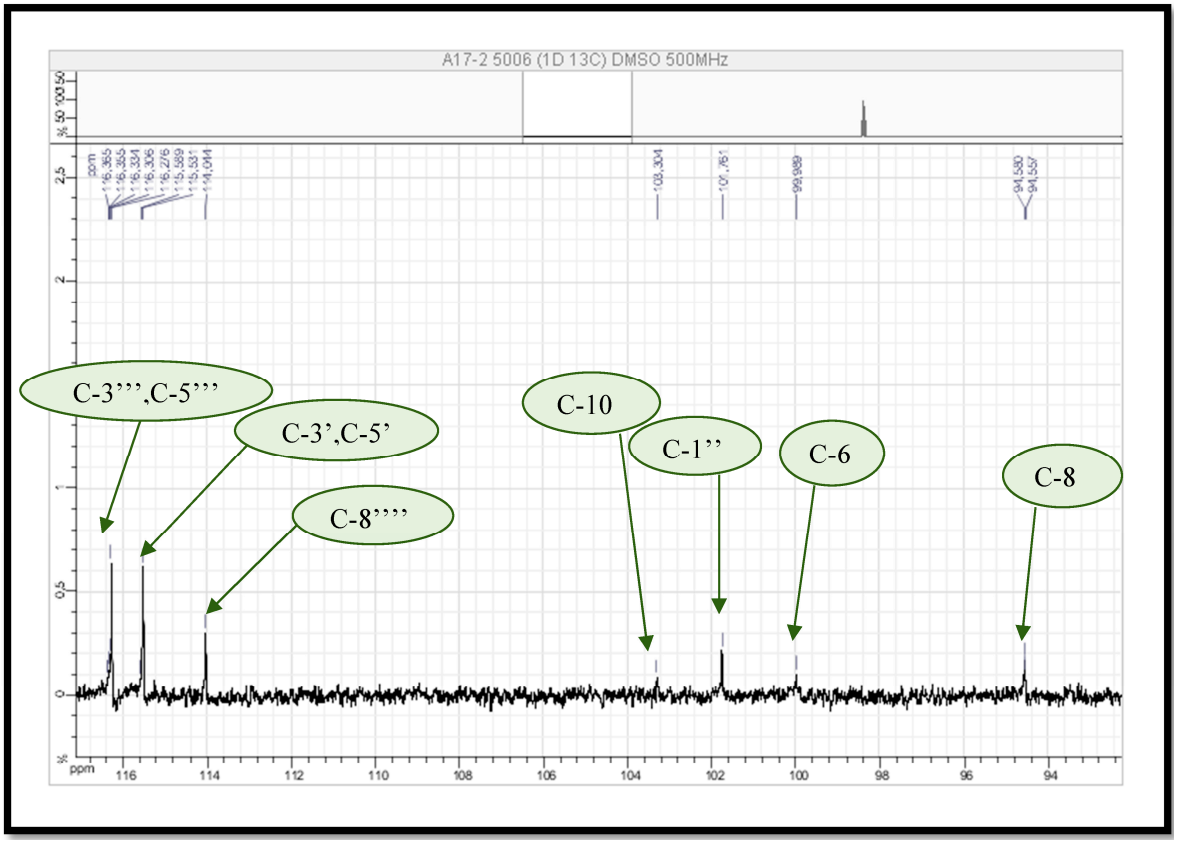


Figure S20. ^{13}C NMR spectrum (spreading out 2) (125 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

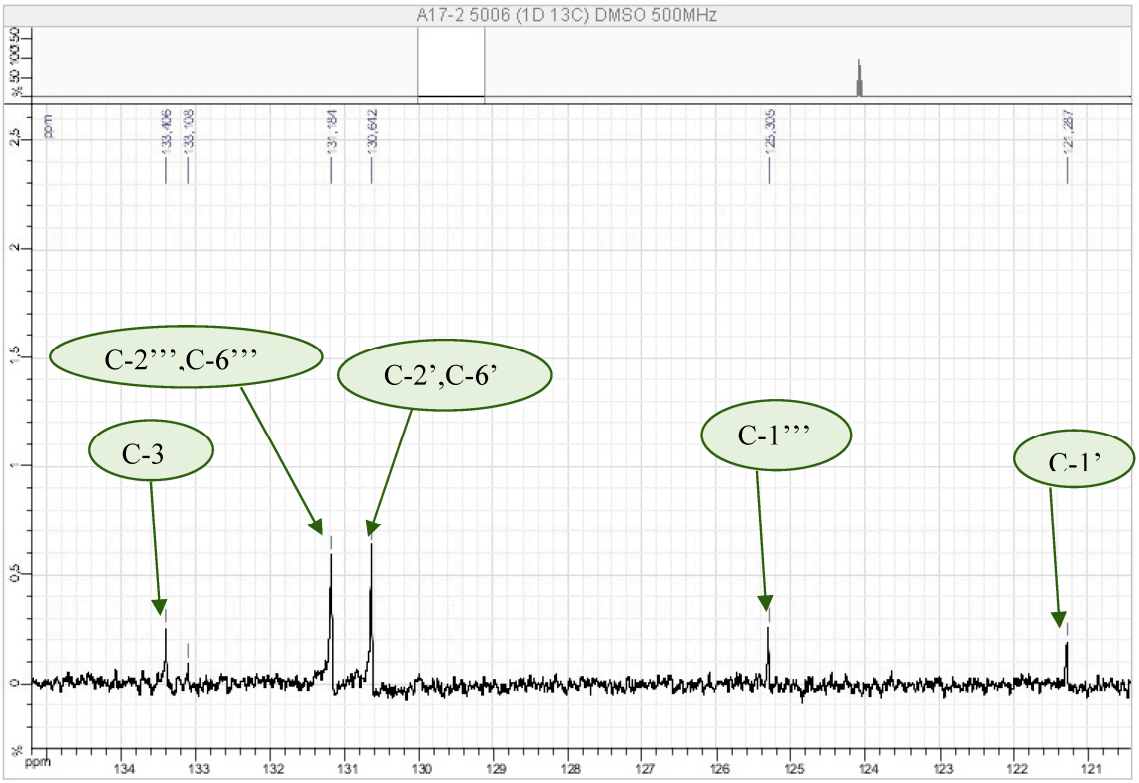


Figure S21. ^{13}C NMR spectrum (spreading out 3) (125 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

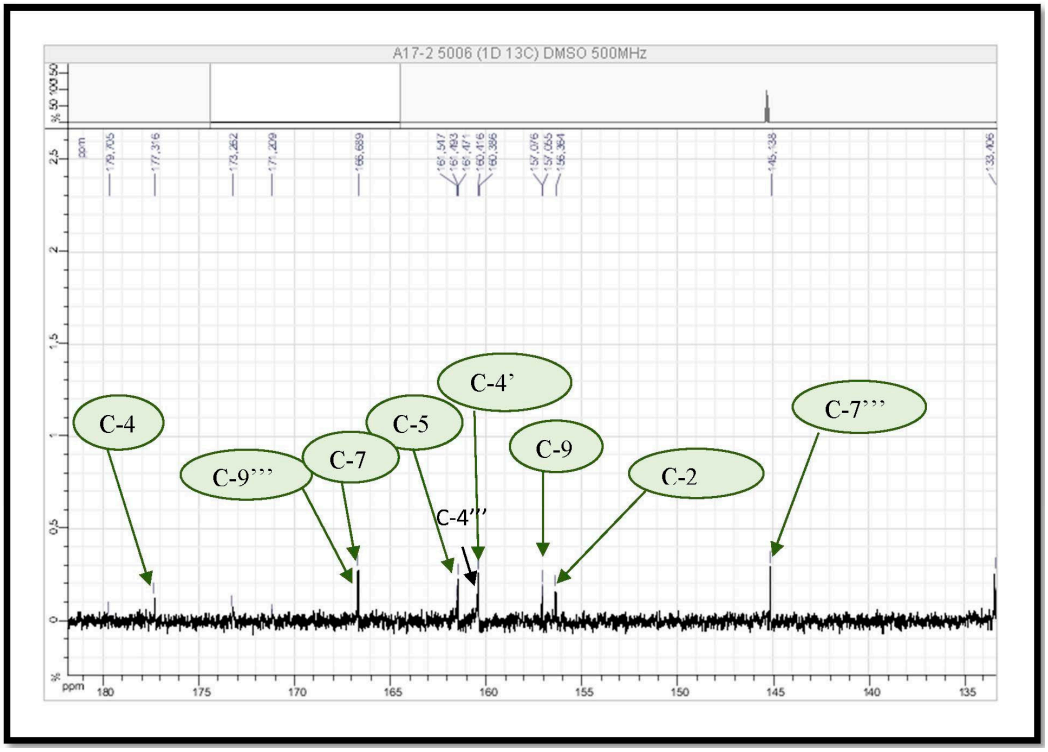
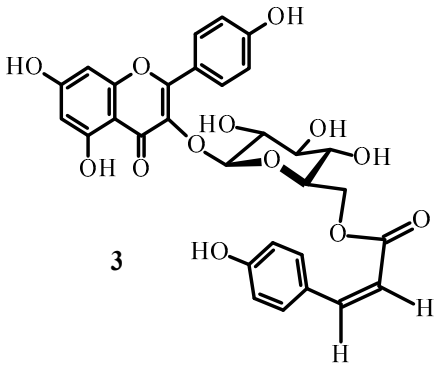


Figure S22. ^{13}C NMR spectrum (spreading out 4) (125 MHz, DMSO-d_6, δppm) of *trans*-tiliroside.

Molecule 3: *Cis*-tiliroside



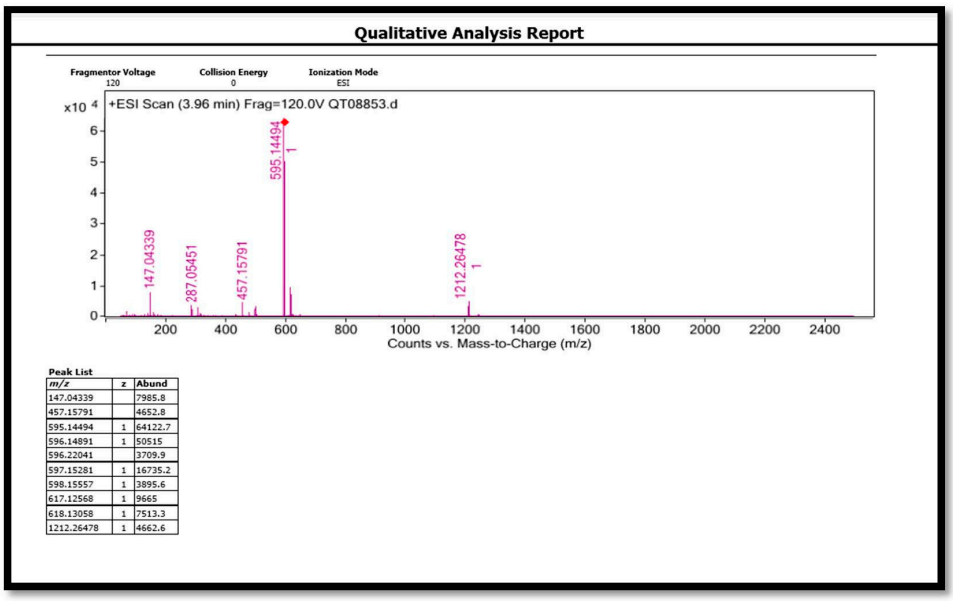


Figure S23. ESI-HRMS(+) of *cis*-tiliroside.

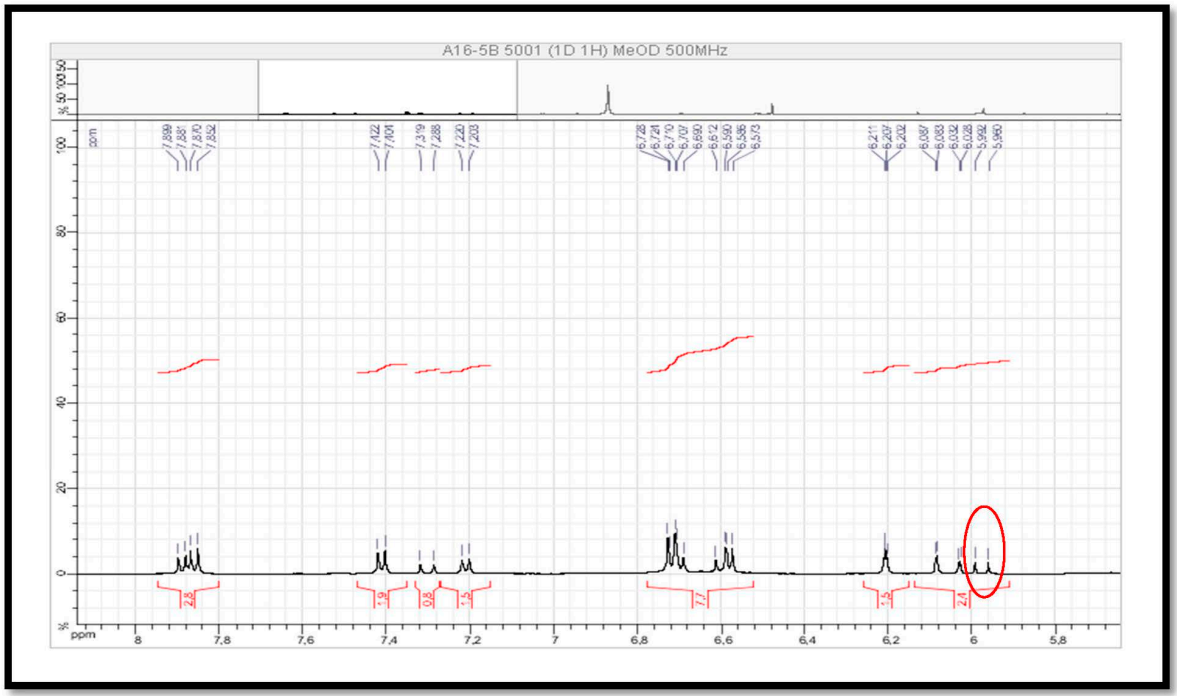


Figure S24. ^{1}H NMR spectrum (spreading out 1) (500 MHz, CD_3OD, δppm) of *cis*-tiliroside.

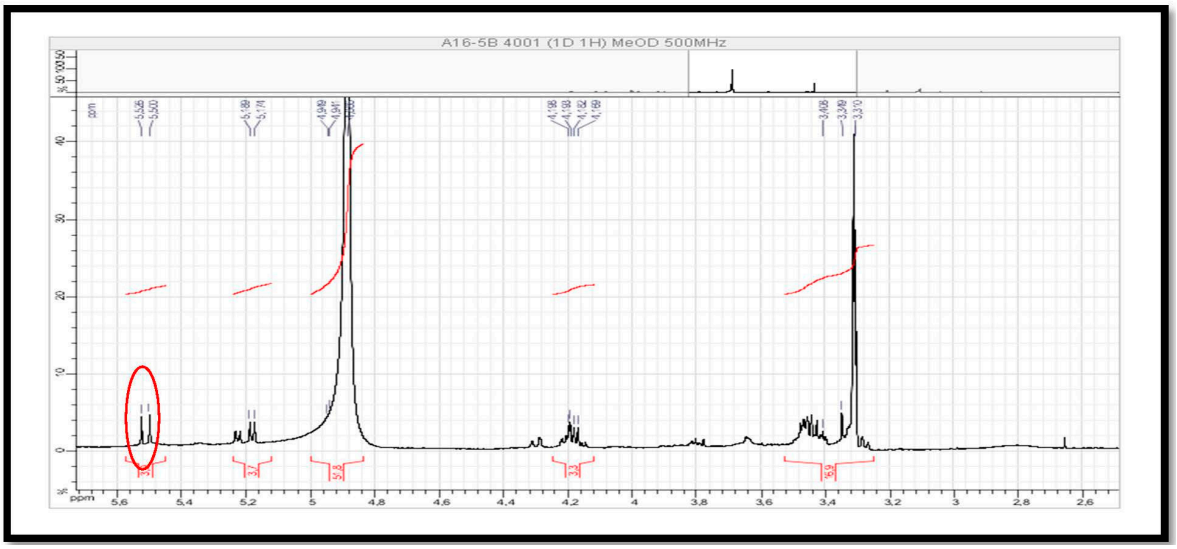


Figure S25. ^{1}H NMR spectrum (spreading out 2) (500 MHz, CD_3OD, δppm) of *cis*-tiliroside.

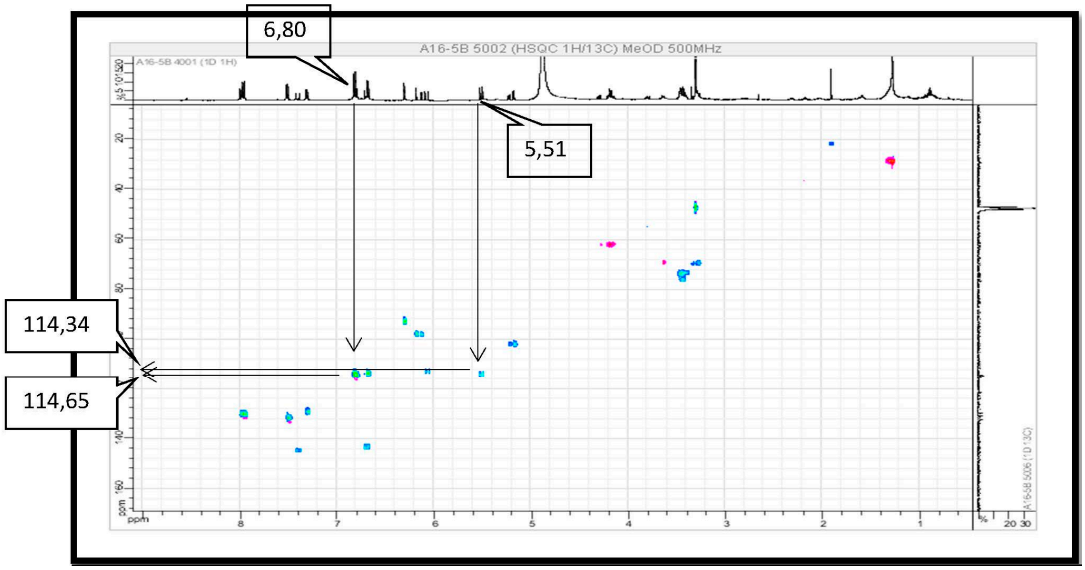
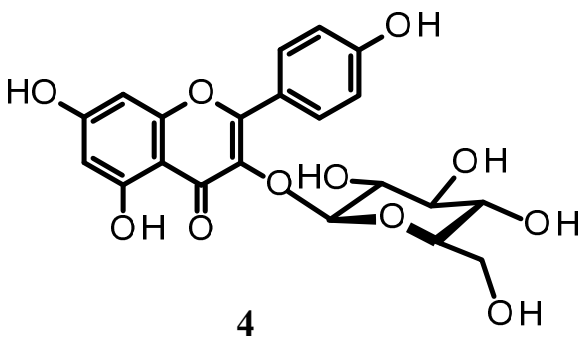


Figure S26. HSQC NMR spectrum (500 MHz, CD_3OD, δppm) of *cis*-tiliroside.

Molecule 4: Astragalin



4

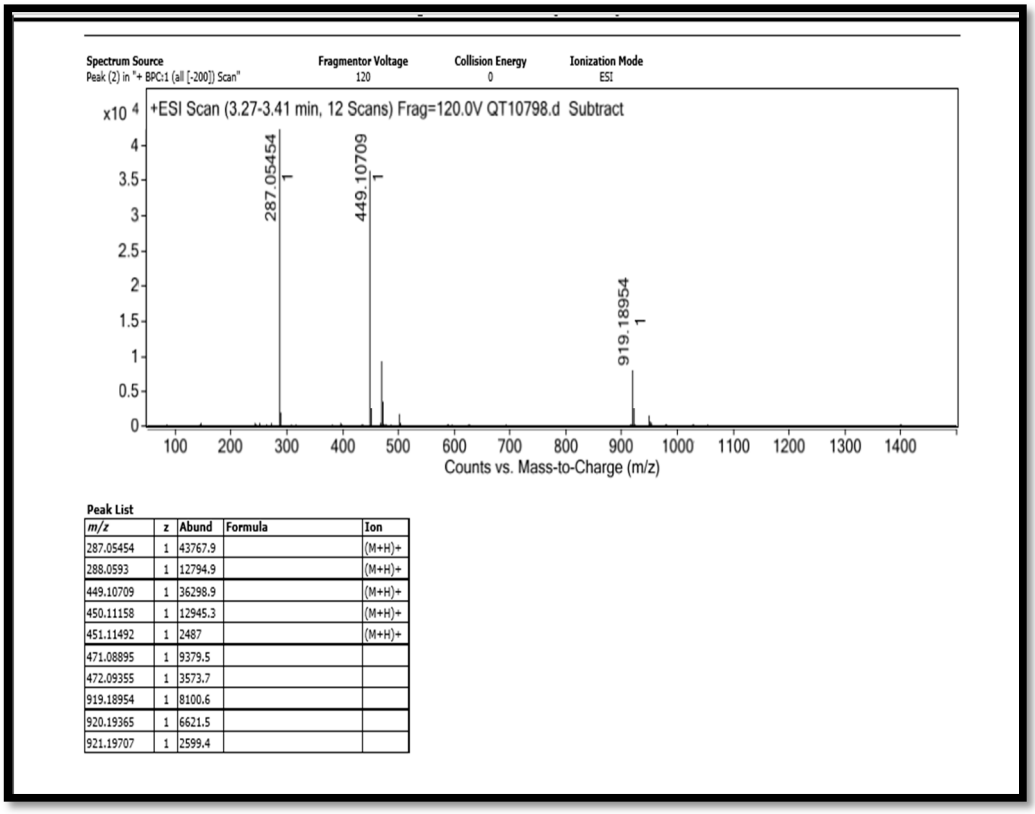


Figure S27. ESI-HRMS(+) of astragalin.

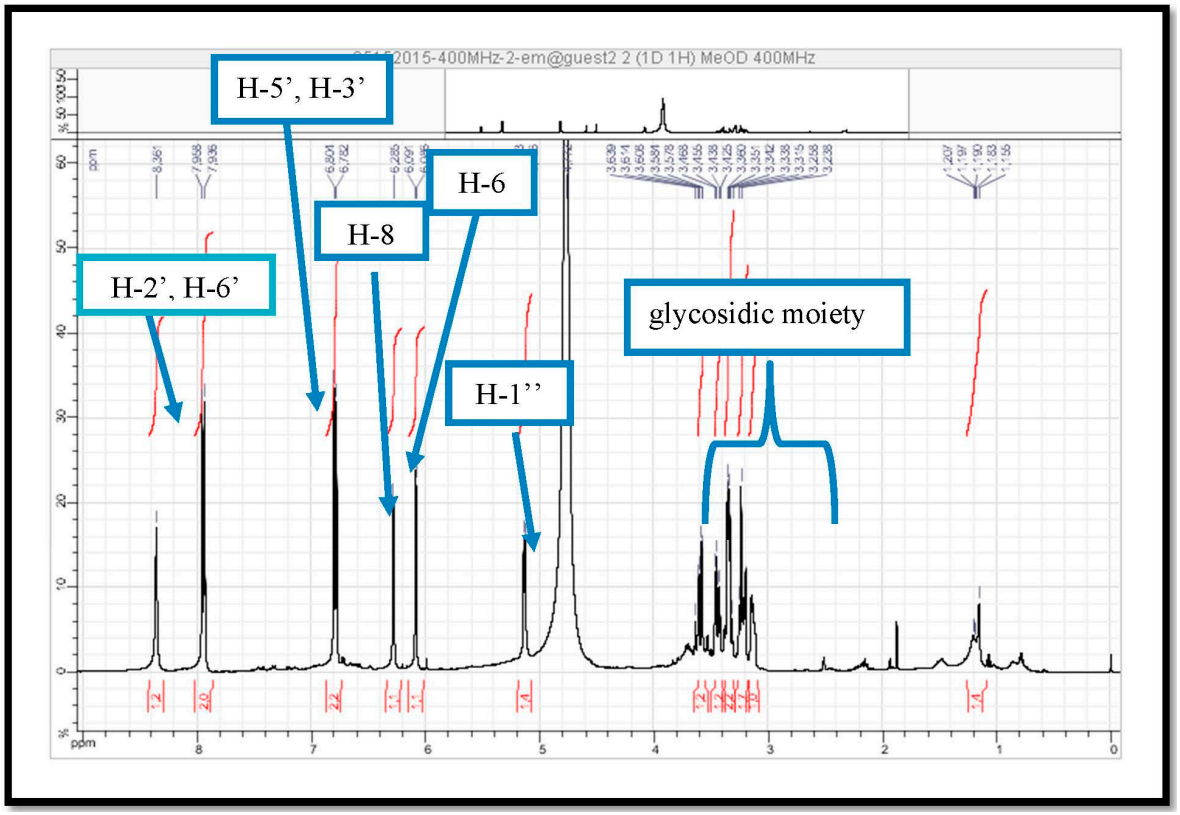


Figure S28. ^{1}H NMR spectrum (400 MHz, CD_3OD, δppm) of astragalin.

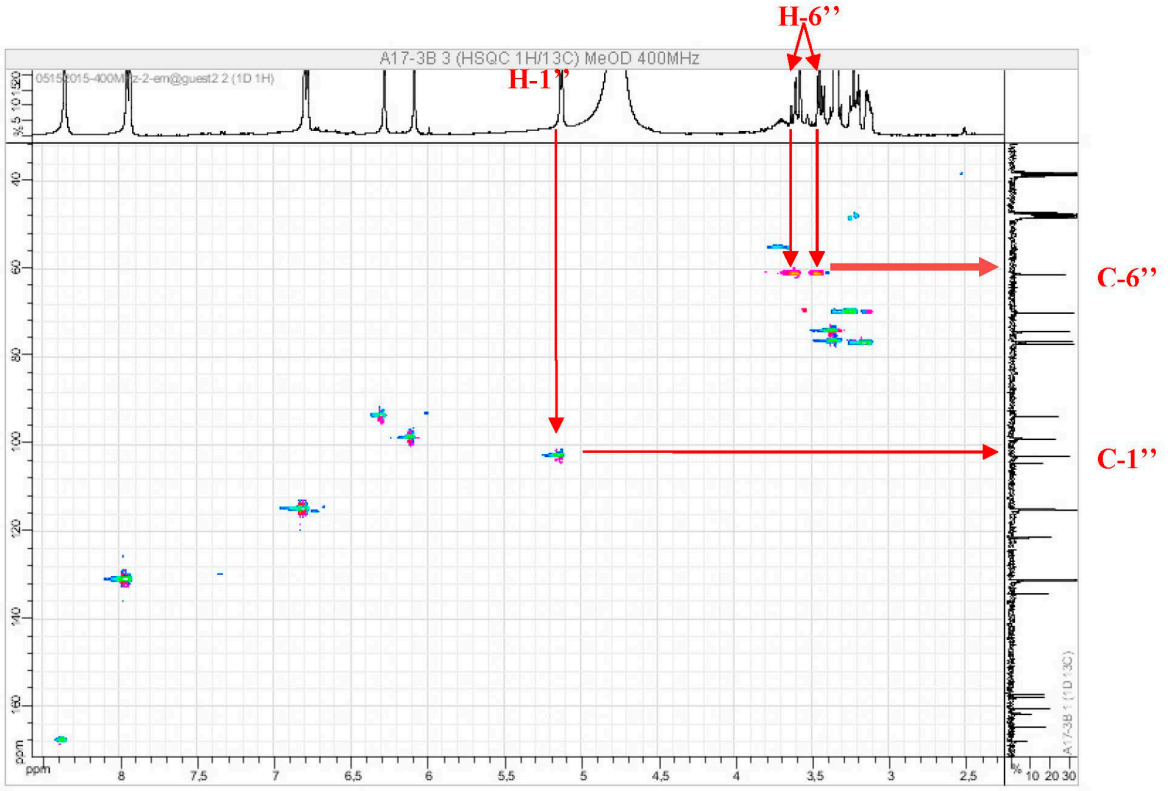


Figure S29. HSQC spectrum (400 MHz, CD_3OD, δppm) of astragalin.

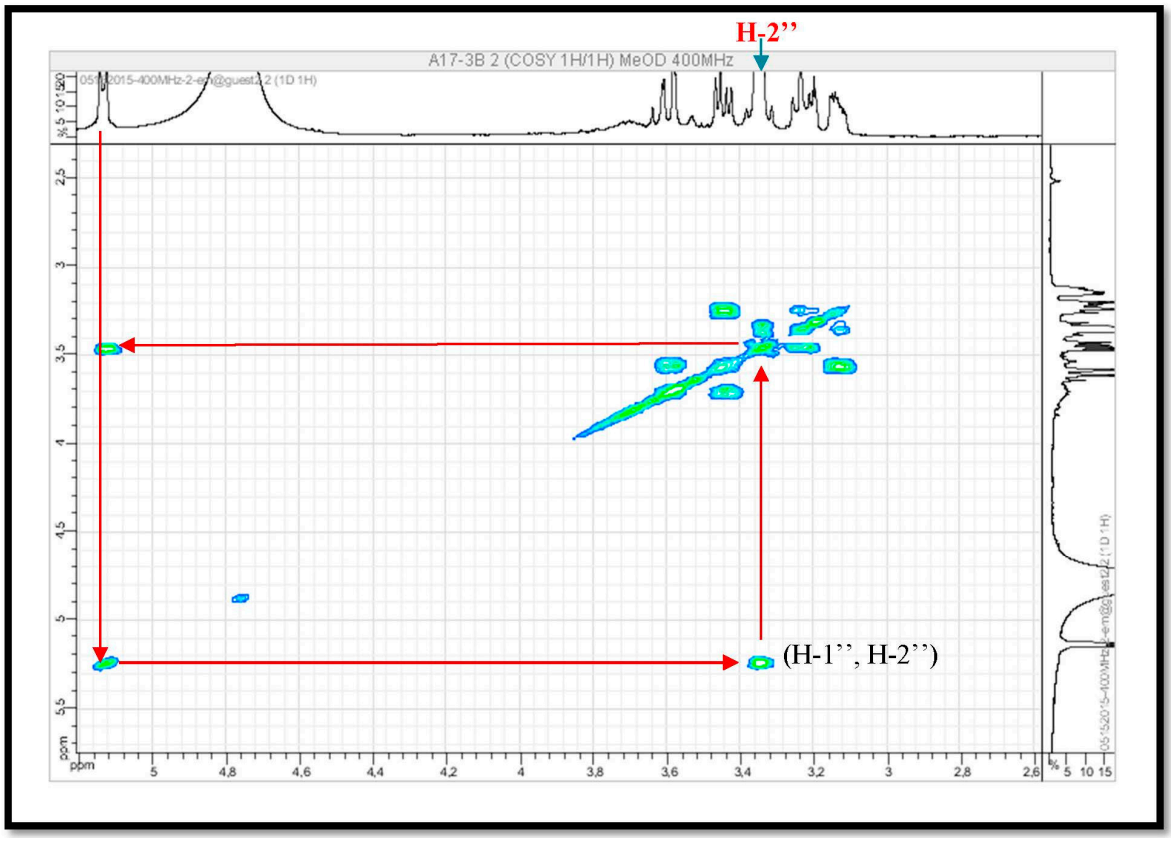


Figure S30. COSY spectrum (400 MHz, CD_3OD, δppm) of astragalin.

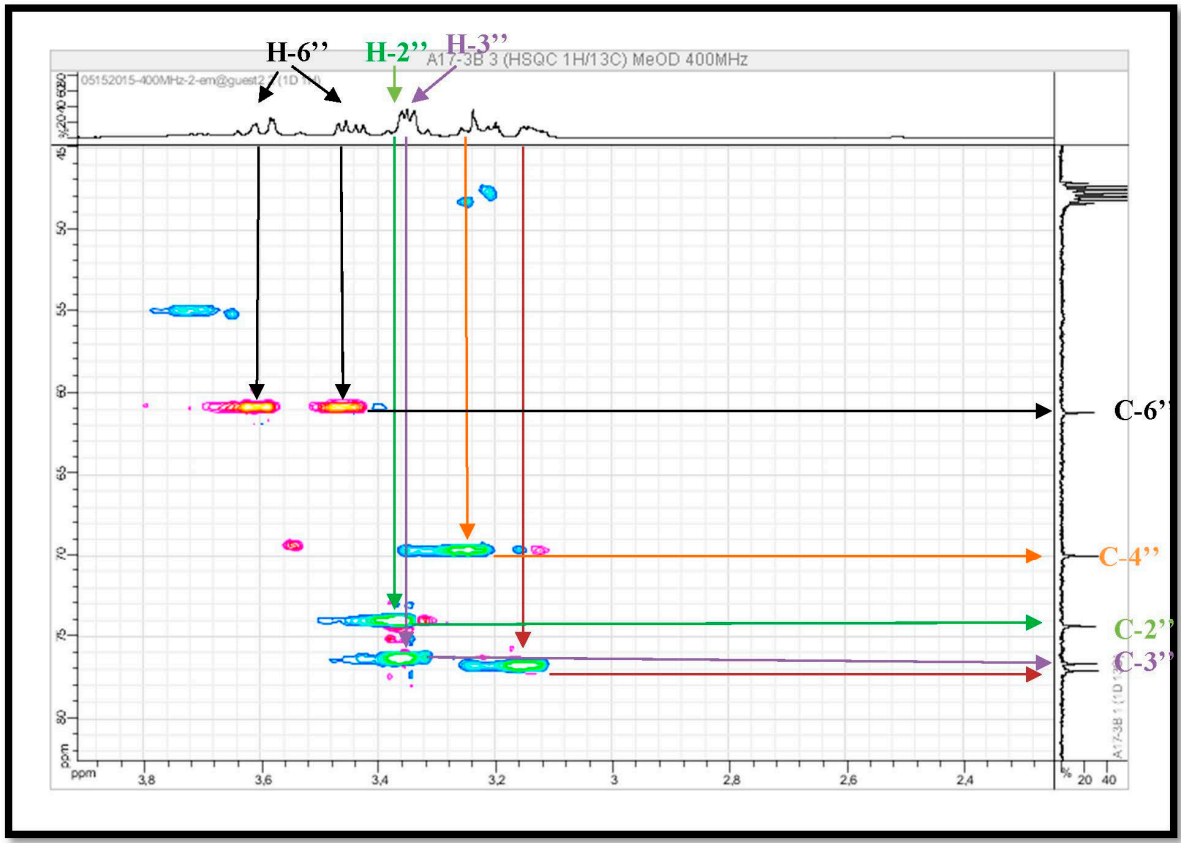


Figure S31. HSQC spectrum (400 MHz, CD_3OD, δppm) of astragalin.

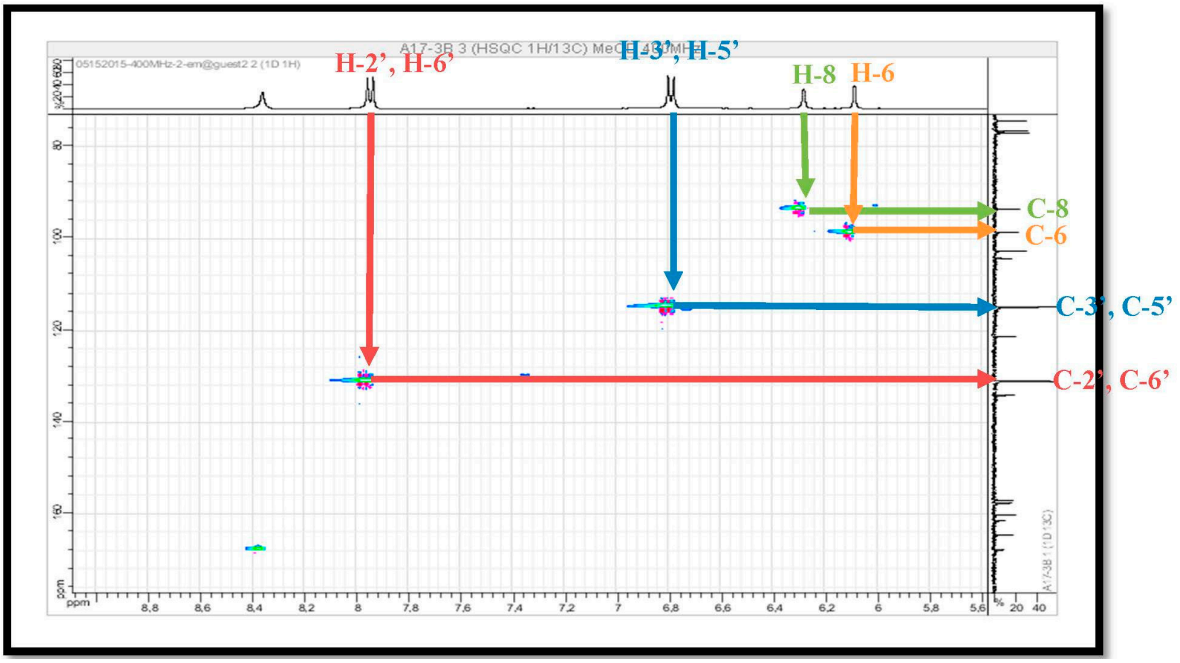


Figure S32. HSQC spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of astragalin.

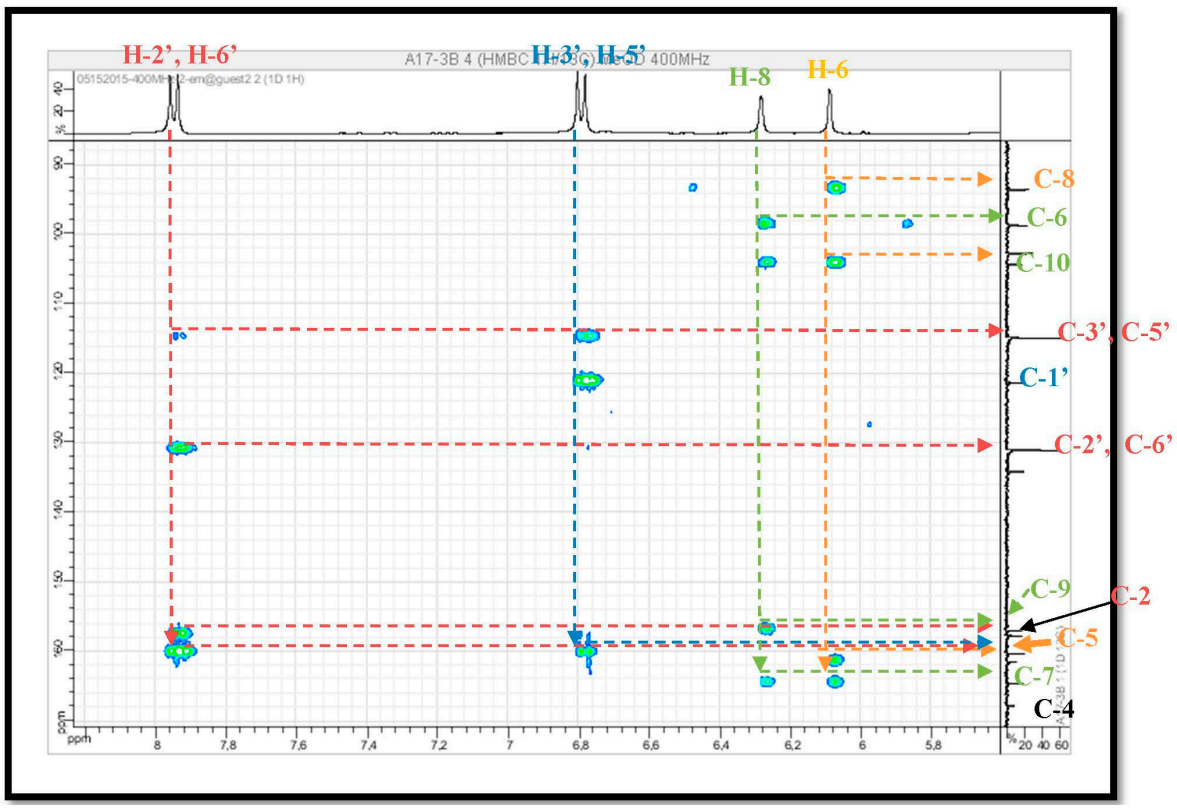


Figure S33. HMBC spectrum (spreading out1) (400 MHz, CD_3OD, δppm) of astragalin.

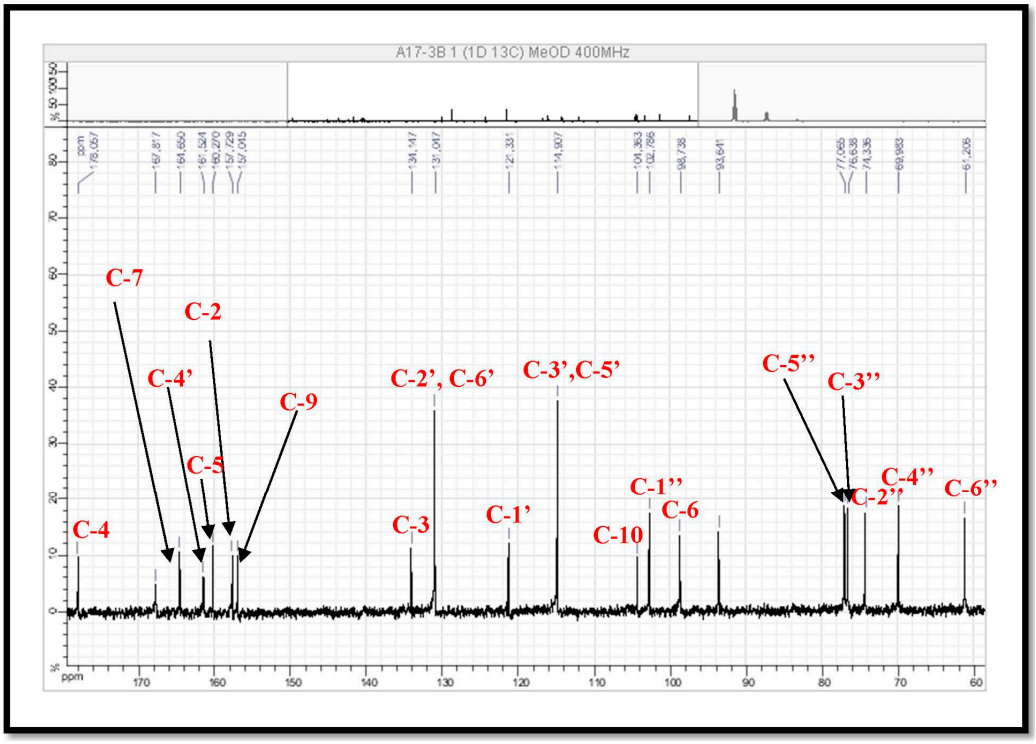
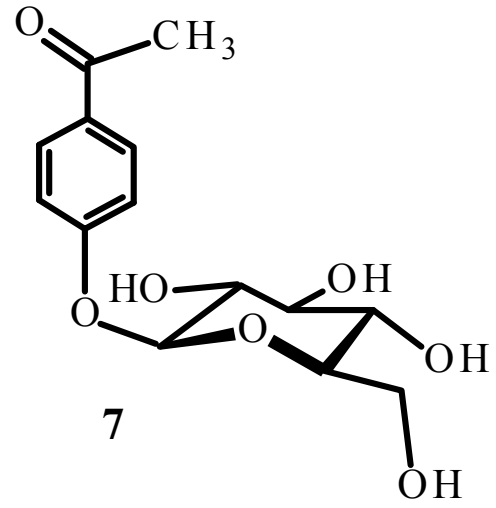


Figure S34. ^{13}C spectrum (100 MHz, CD_3OD, δppm) of astragalin.

Molecule 7: Picein



7

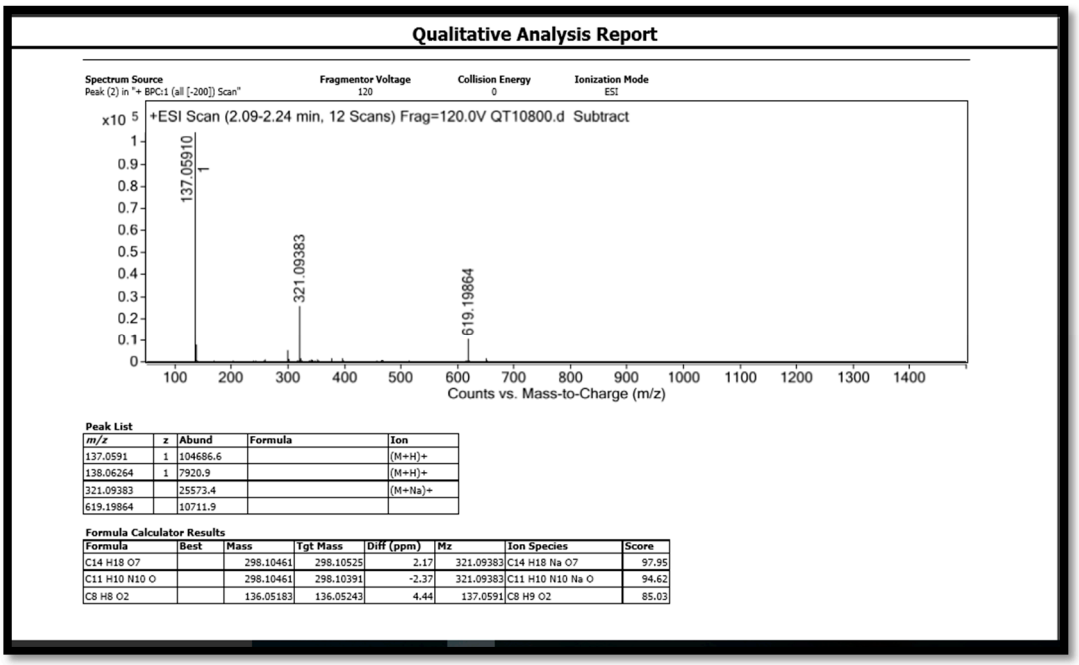


Figure S35. ESI-HRMS(+) of picein.

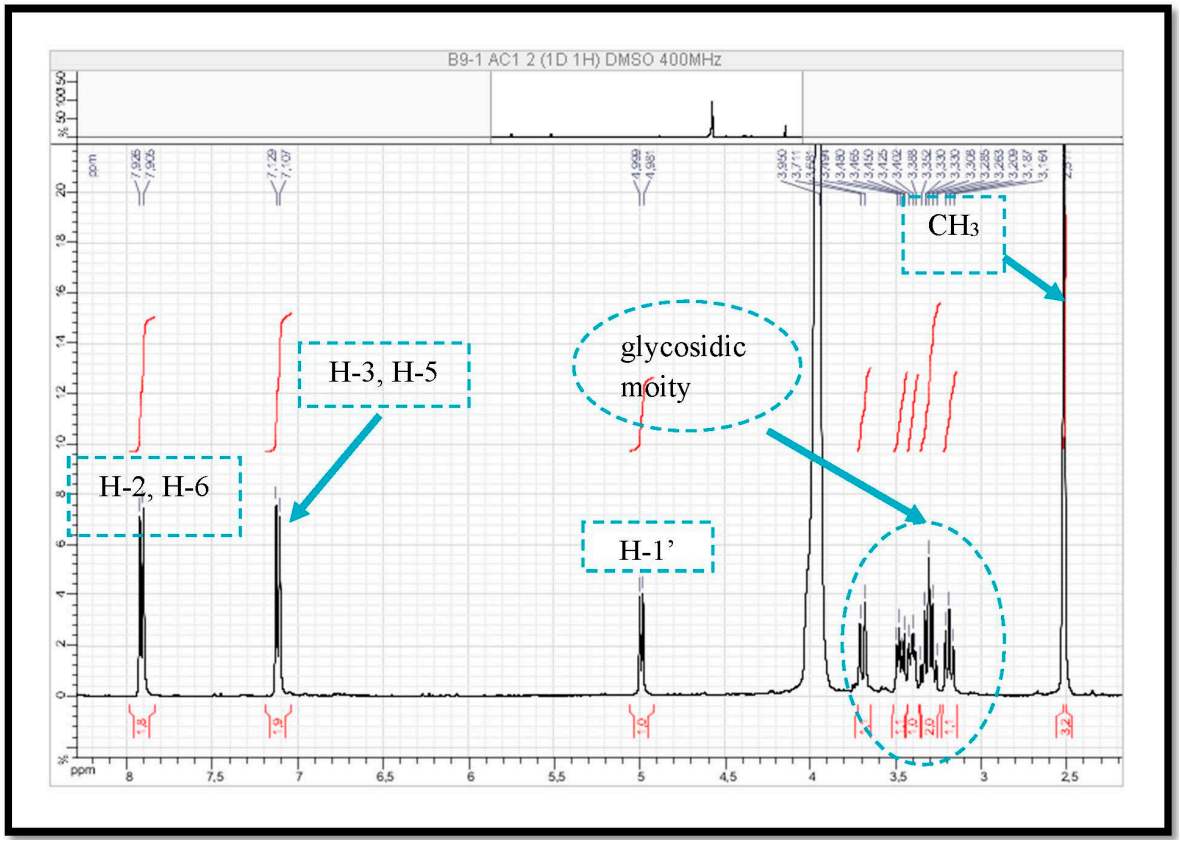


Figure S36. ^{1}H NMR spectrum (spreading out1) (400 MHz, DMSO-d_6, δppm) of picein.

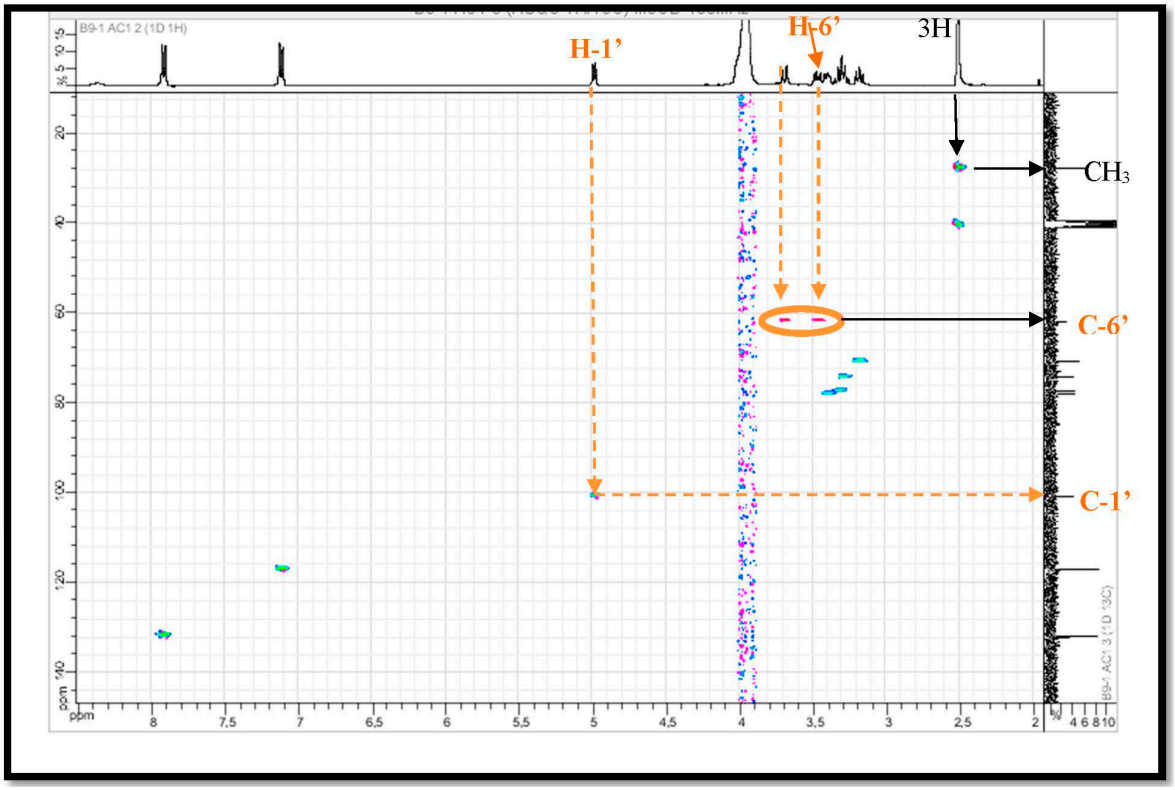


Figure S37. HSQC NMR spectrum (400 MHz, DMSO-d_6, δppm) of picein.

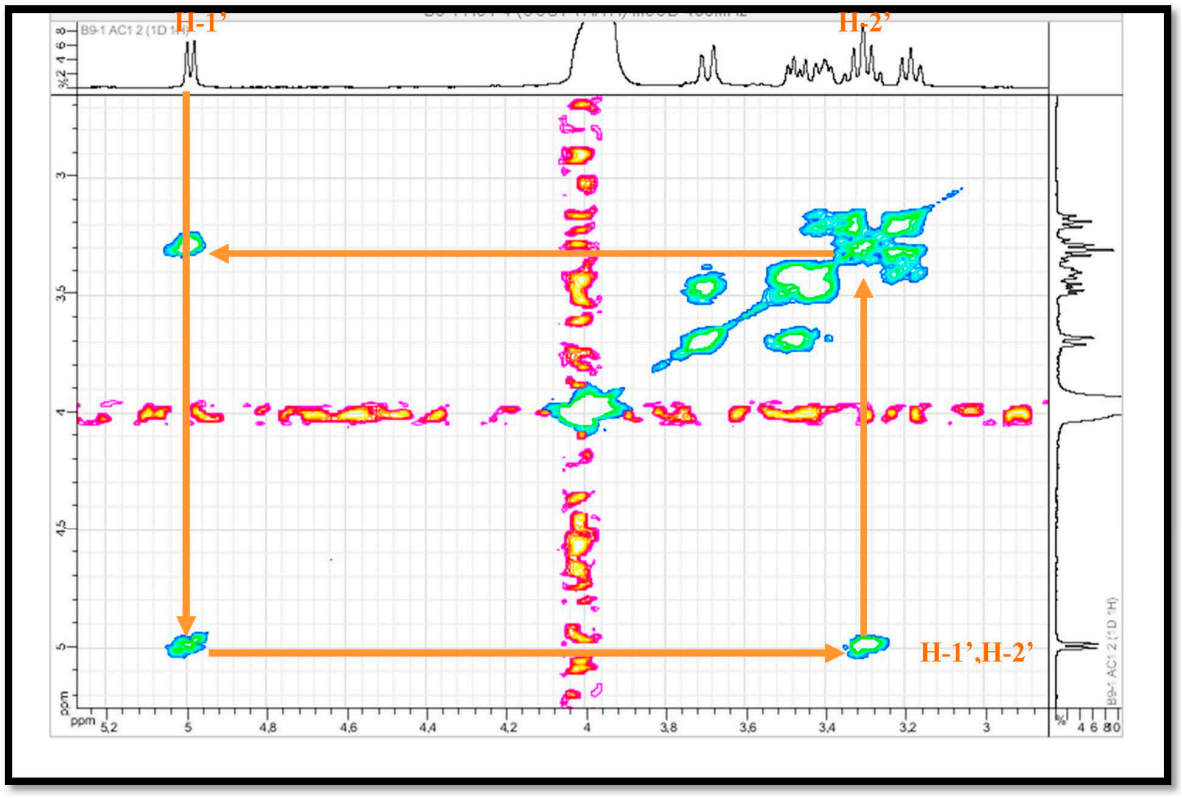


Figure S38. COSY NMR spectrum (400 MHz, DMSO-d_6, δppm) of picein.

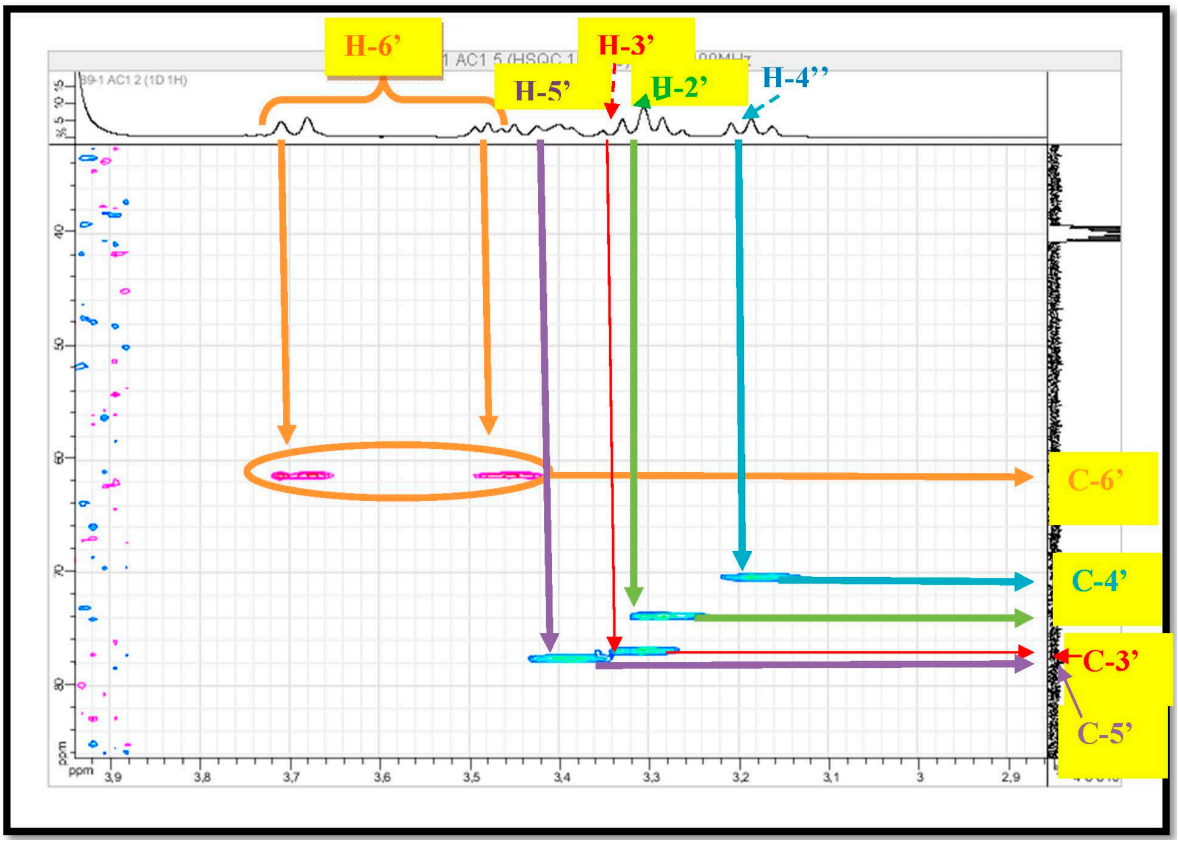


Figure S39. HSQC NMR spectrum (spreading out 1) (400 MHz, DMSO-d_6, δppm) of picein.

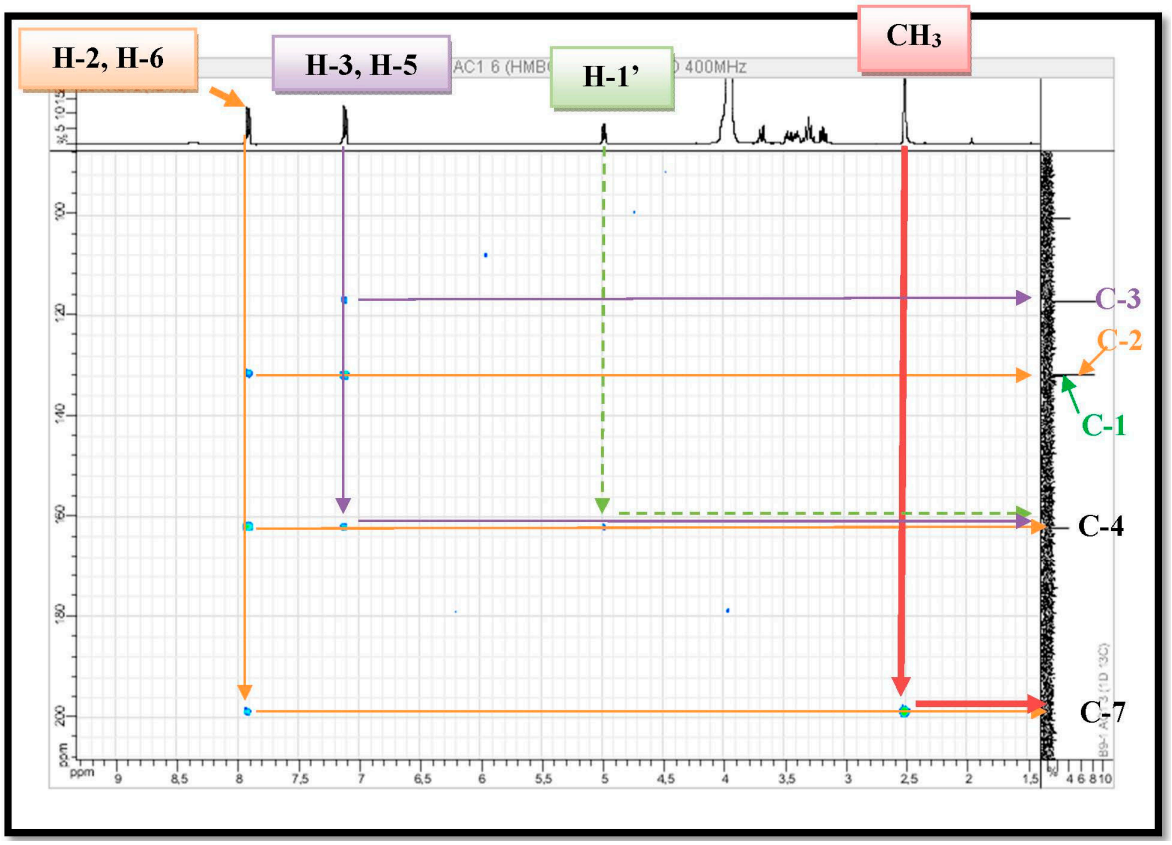


Figure S40. HMBC NMR spectrum (400 MHz, DMSO-d_6, δppm) of picein.

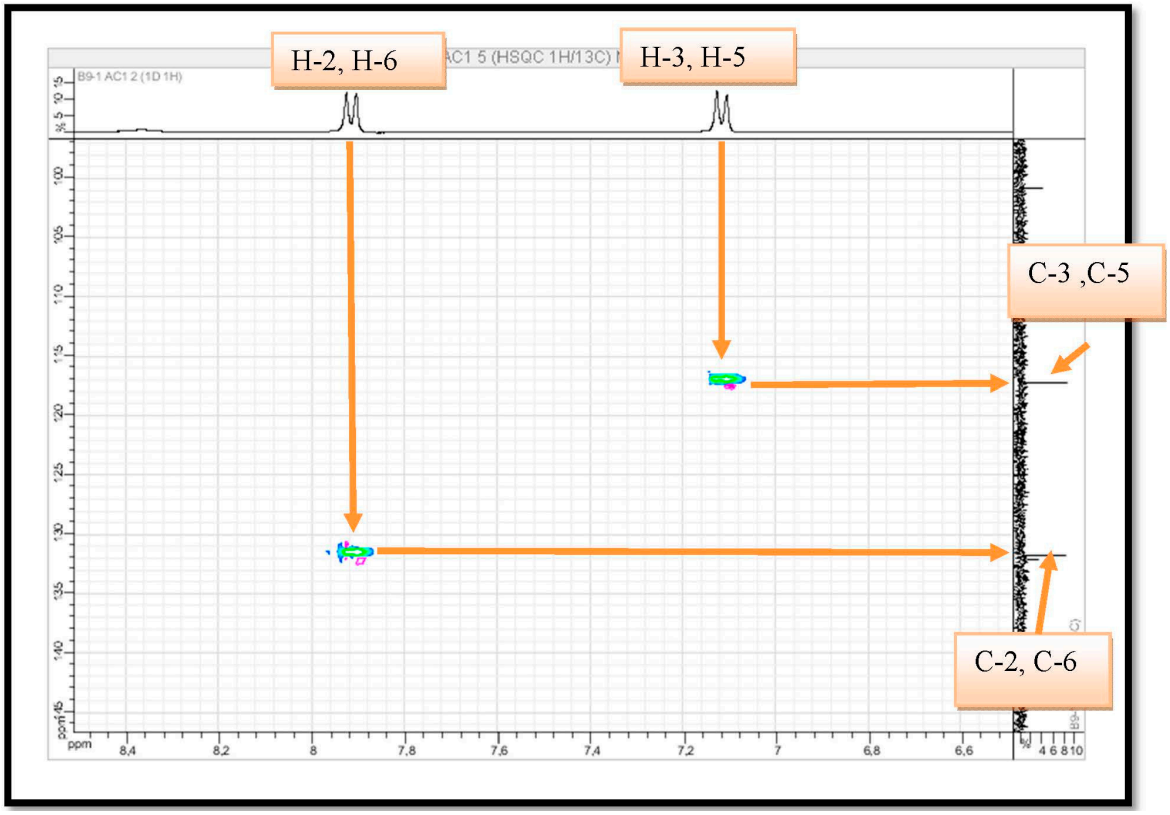


Figure S41. HSQC NMR spectrum (spreading out 2) (400 MHz, DMSO-d_6, δppm) of picein.

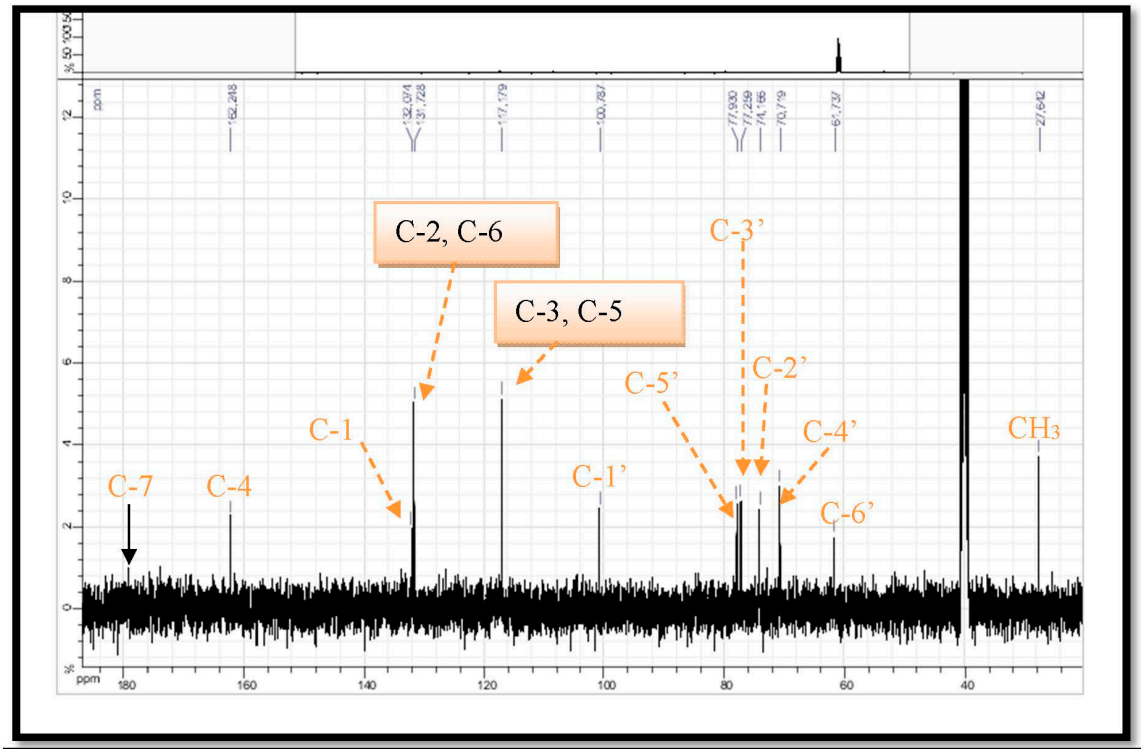
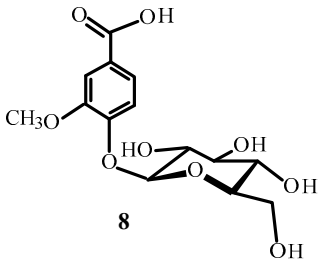


Figure S42. ^{13}C NMR spectrum (spreading out 1) (100 MHz, DMSO-d_6, δppm) of picein.

Molecule 8: Vanillic acid 4-*O*-β-D-glucopyranoside



8

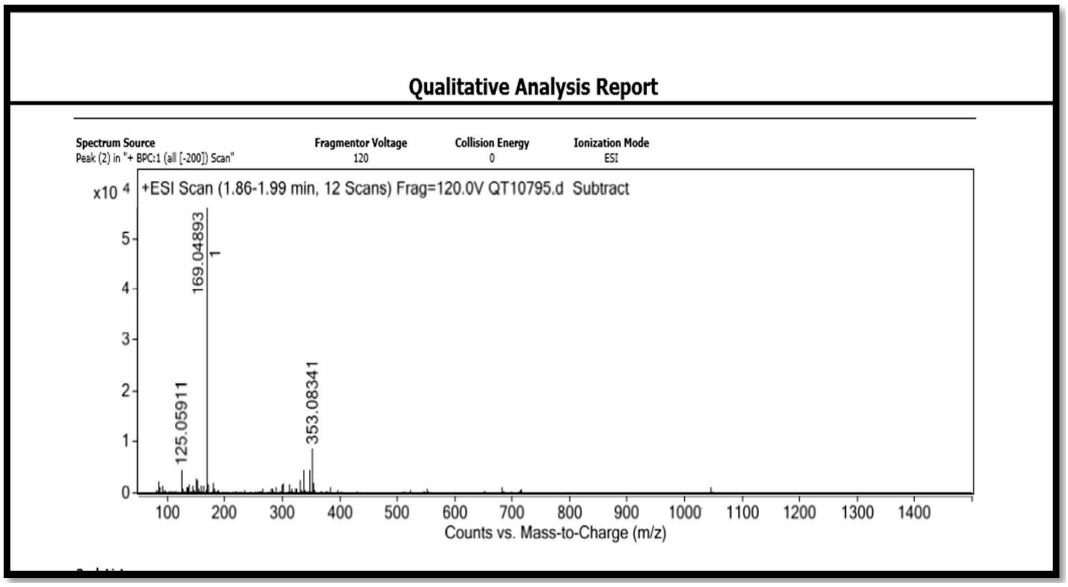


Figure S43. ESI-HRMS(+) of Vanillic acid 4-*O*-β-D-glucopyranoside.

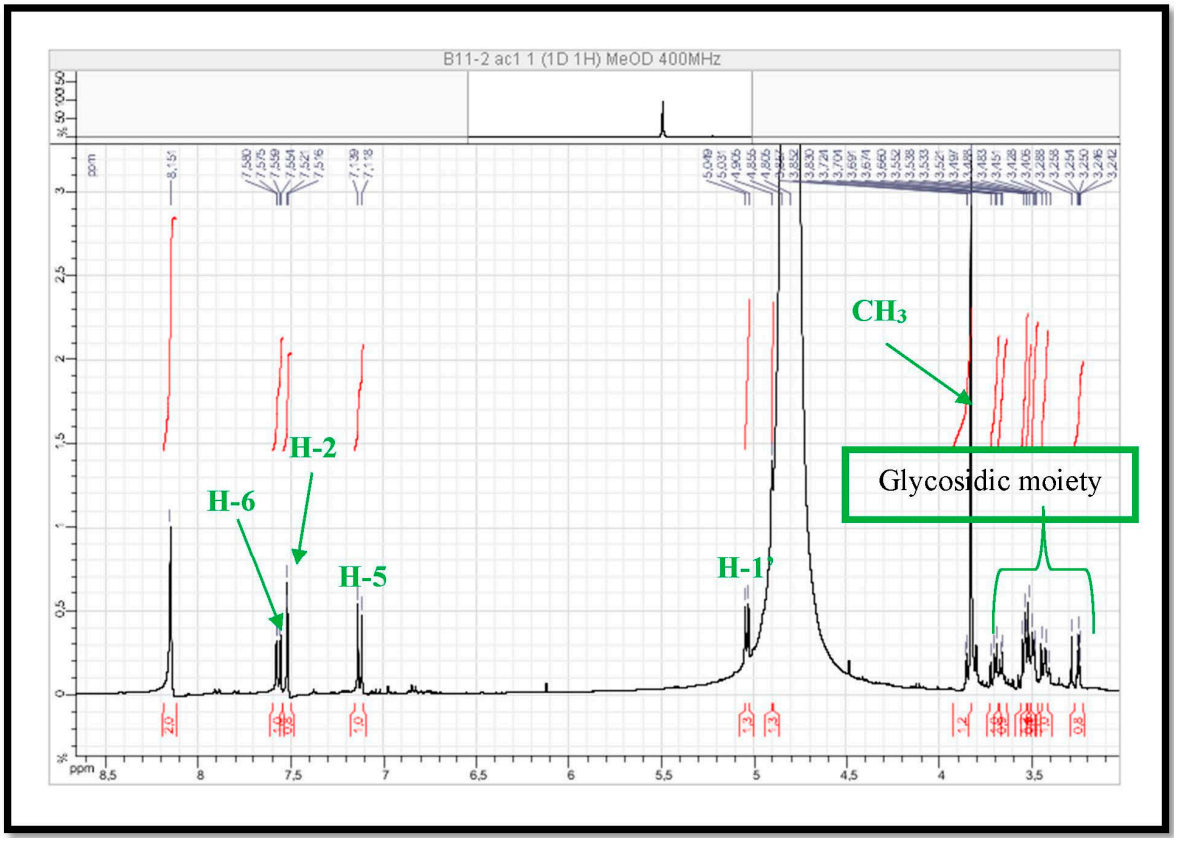


Figure S44. ^{1}H NMR spectrum (400 MHz, CD_3OD, δppm) of Vanillic acid 4-*O*-β-D-glucopyranoside.

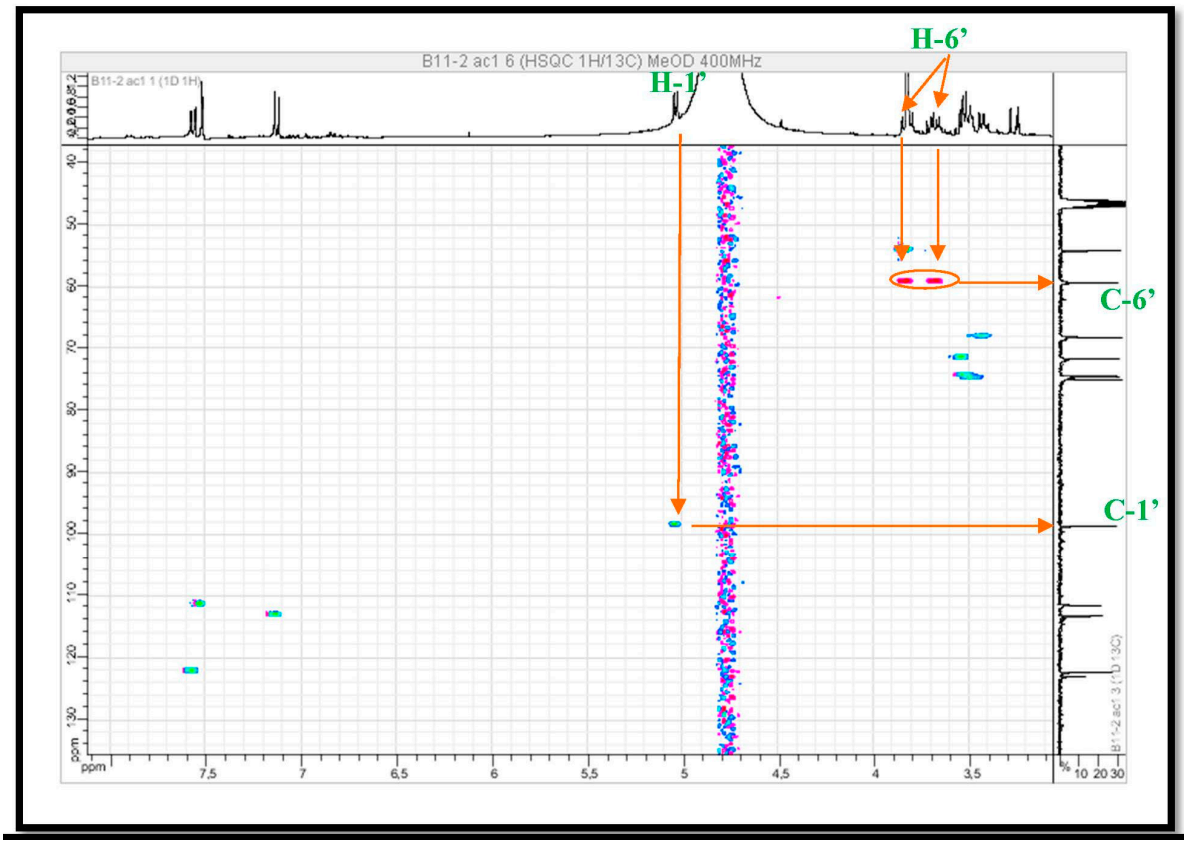


Figure S45. HSQC NMR spectrum (400 MHz, CD_3OD, δppm) of Vanillic acid 4-*O*-β-D-glucopyranoside.

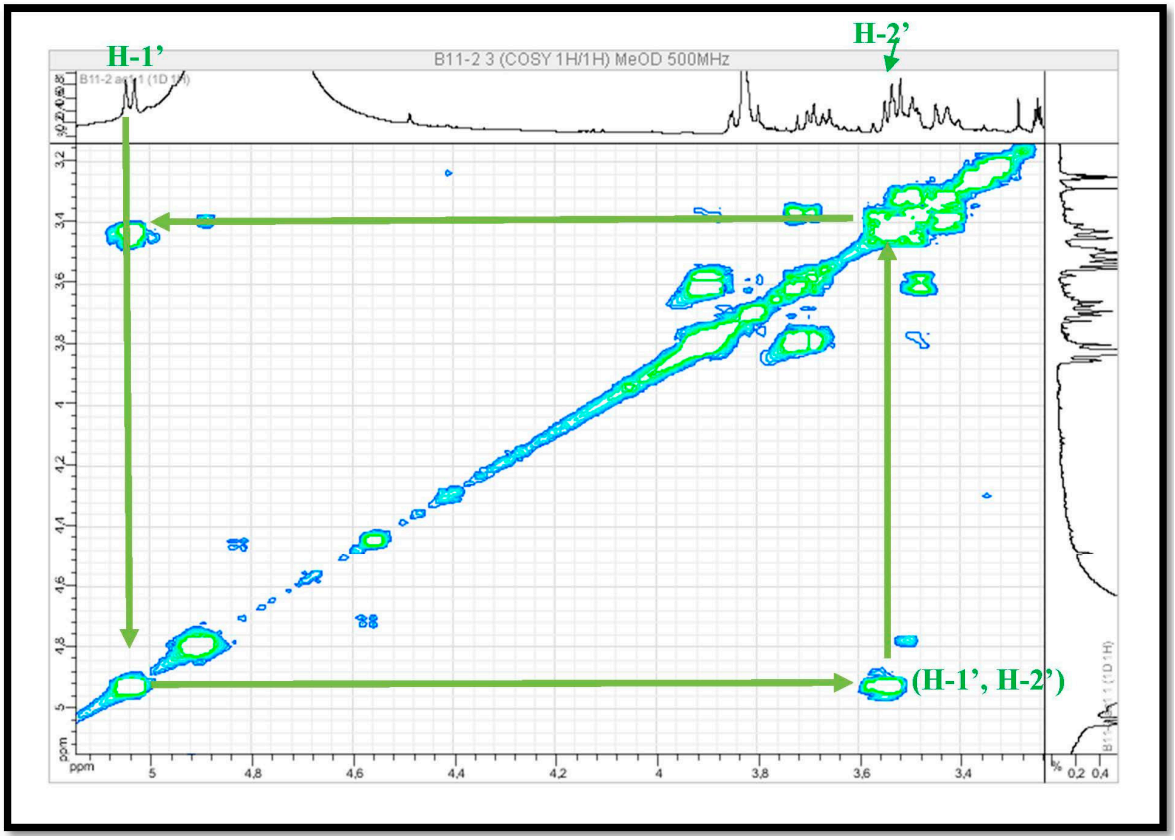


Figure S46. COSY NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of Vanillic acid 4-*O*-β-D-glucopyranoside.

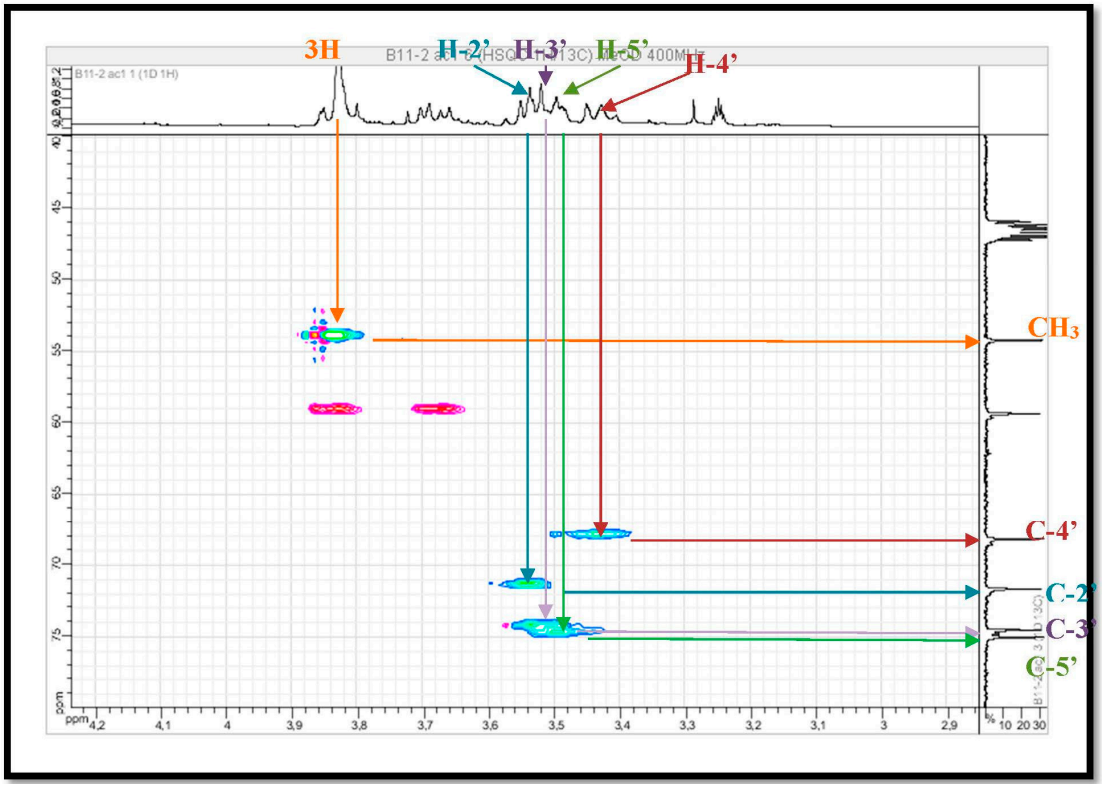


Figure S47. HSQC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of Vanillic acid 4-*O*-β-D-glucopyranoside.

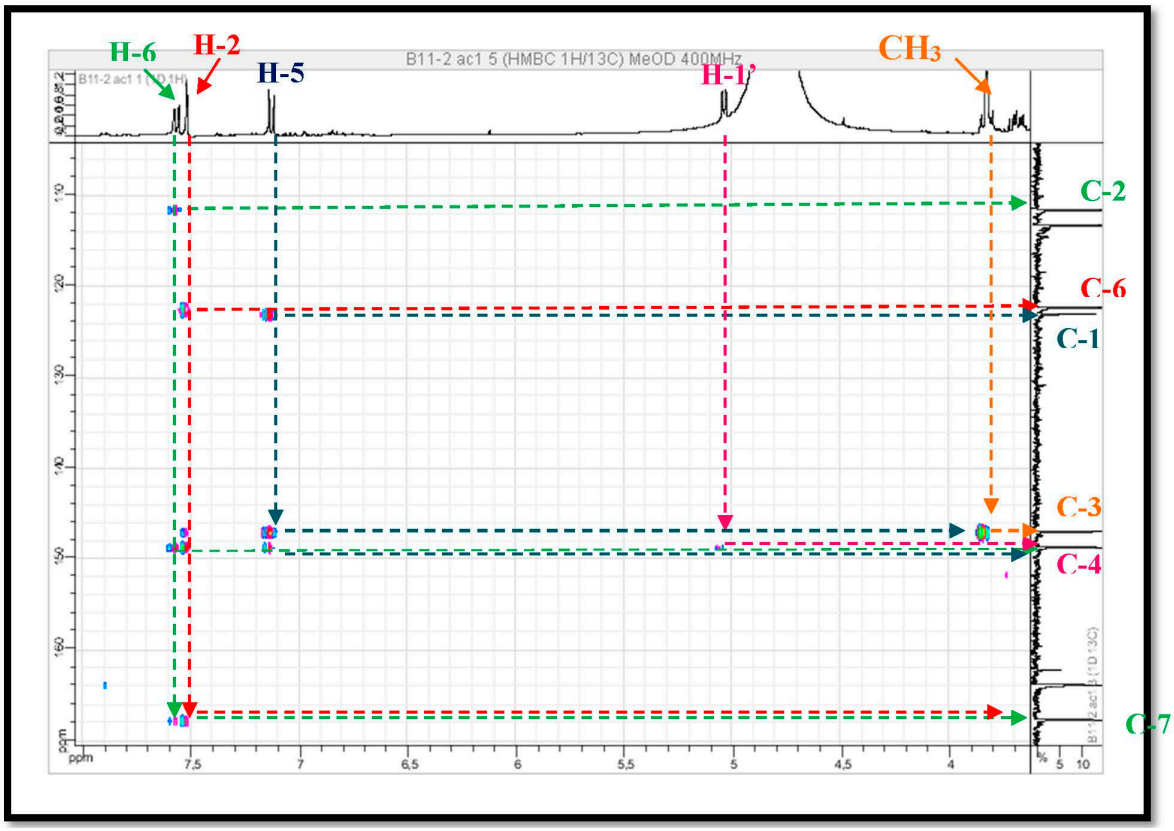


Figure S48. HMBC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of Vanillic acid 4-*O*-β-D-glucopyranoside.

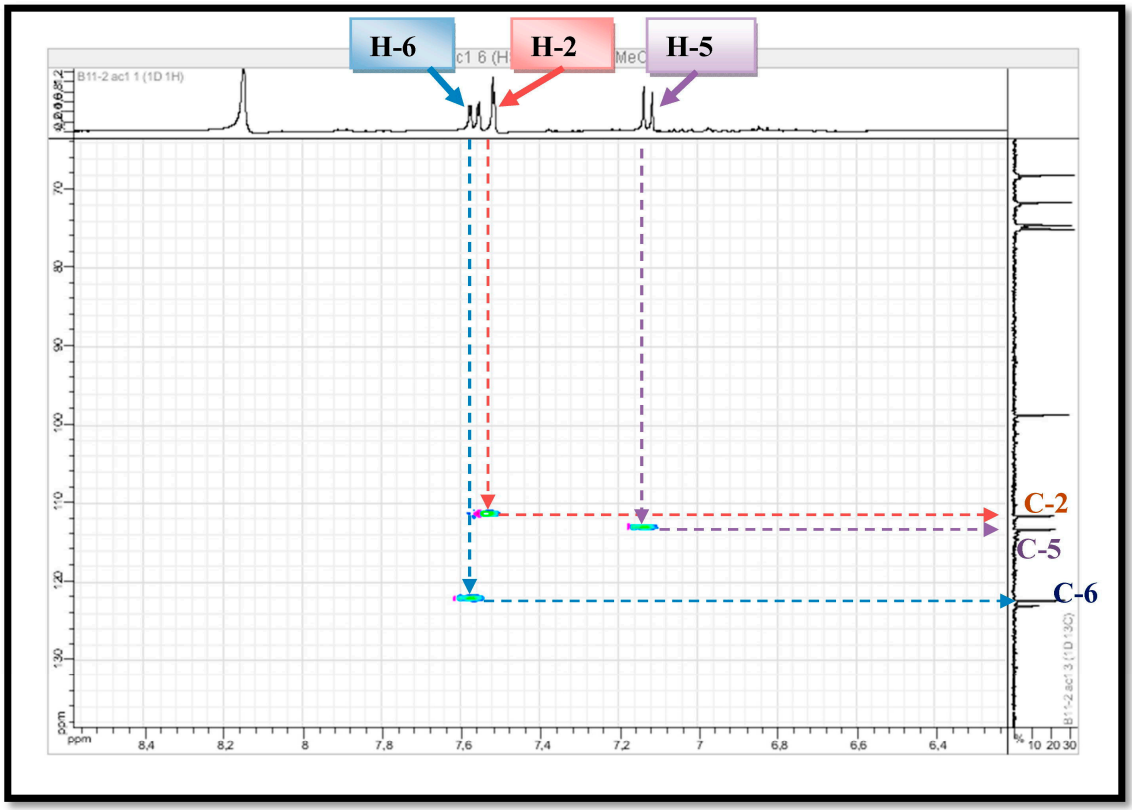


Figure S49. HSQC NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of Vanillic acid 4-*O*-β-D-glucopyranoside.

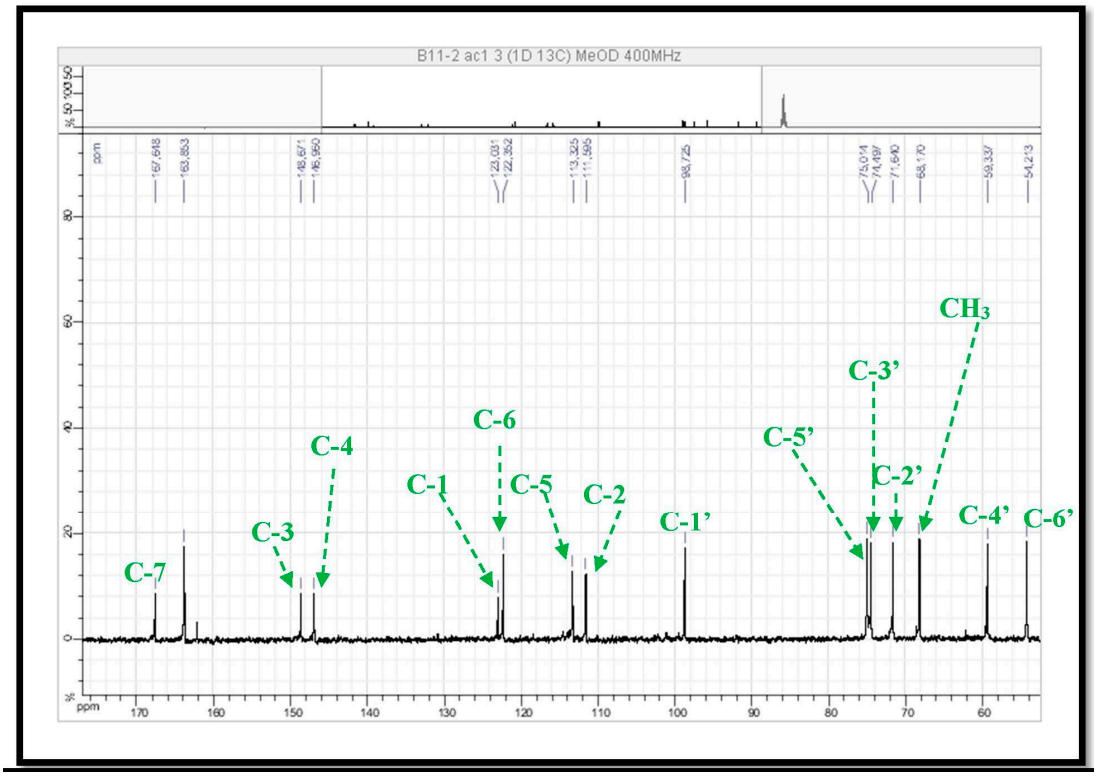
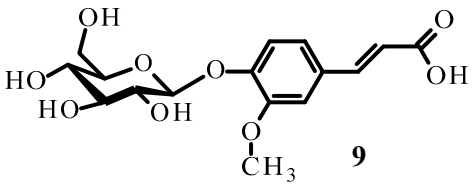


Figure S50. ^{13}C NMR spectrum (100 MHz, CD_3OD, δppm) of vanillic acid 4-*O*-β-D-glucopyranoside.

Molecule 9: Lavandoside



9

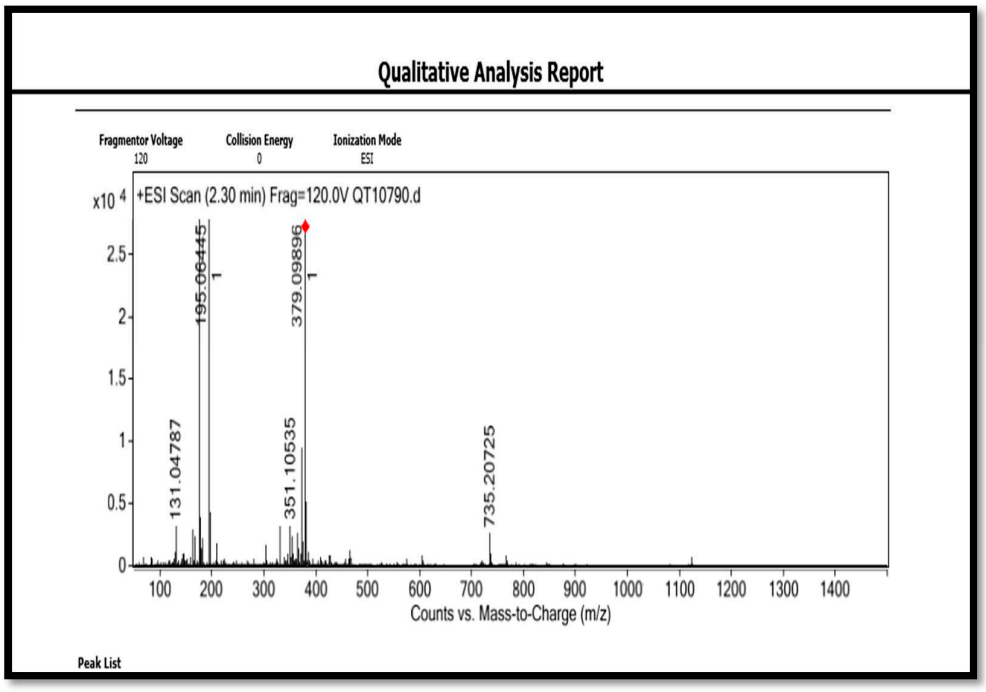


Figure S51. ESI-HRMS(+) of lavandoside.

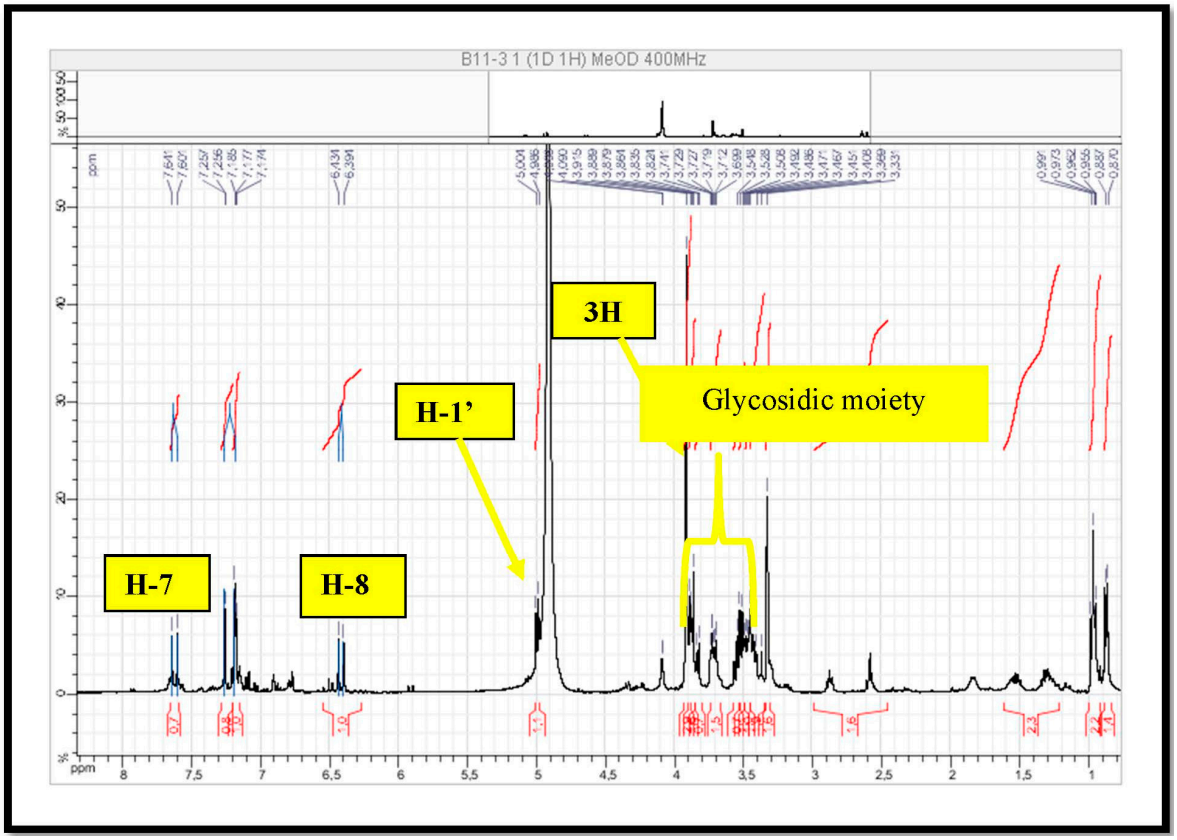


Figure S52. ^{1}H NMR spectrum (400 MHz, CD_3OD, δppm) of lavandoside.

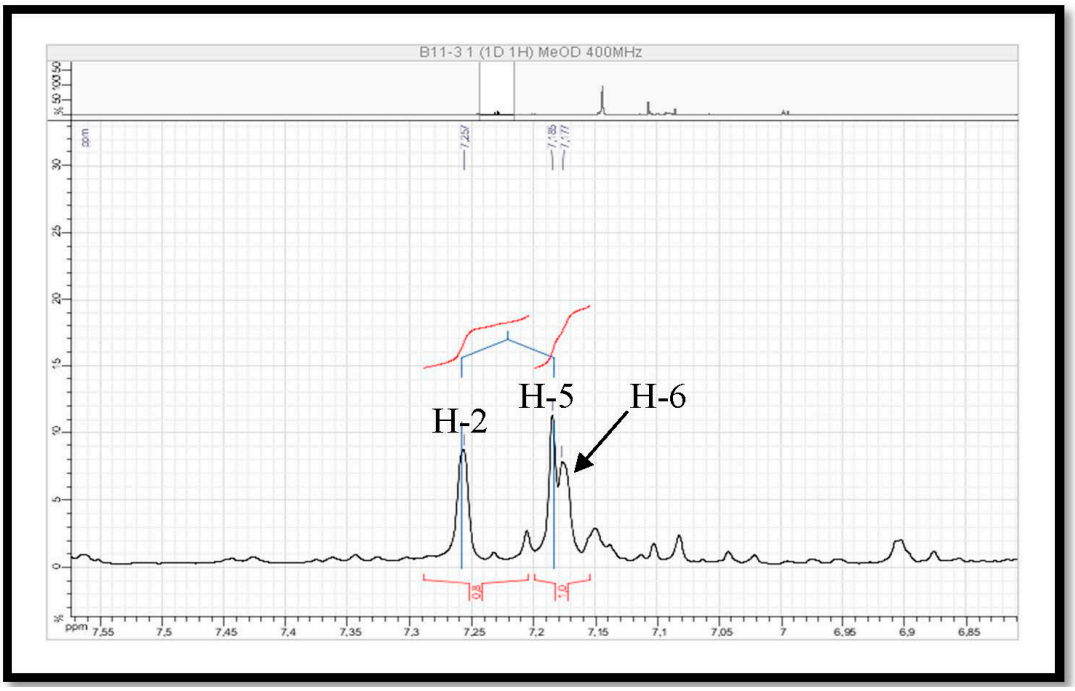


Figure S53. ^{1}H NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of lavandoside.

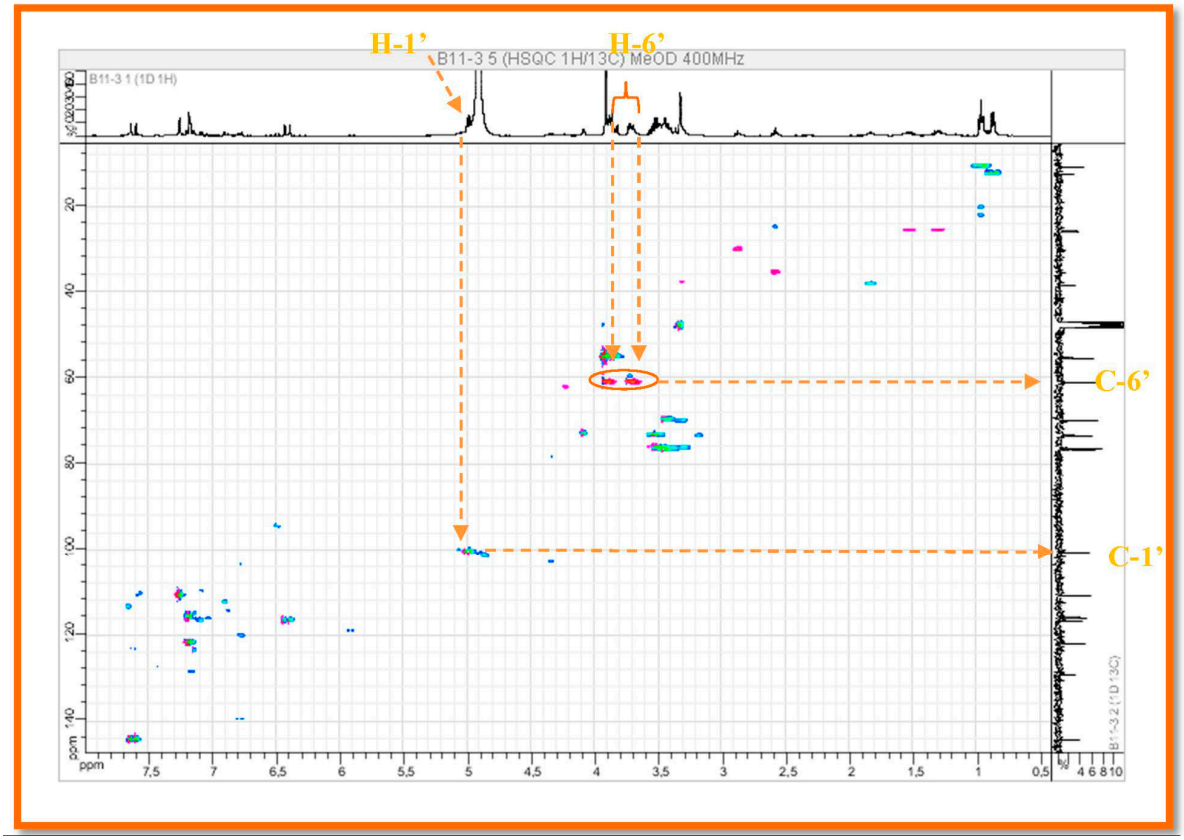


Figure S54. HSQC NMR spectrum (400 MHz, CD_3OD, δppm) of lavandoside.

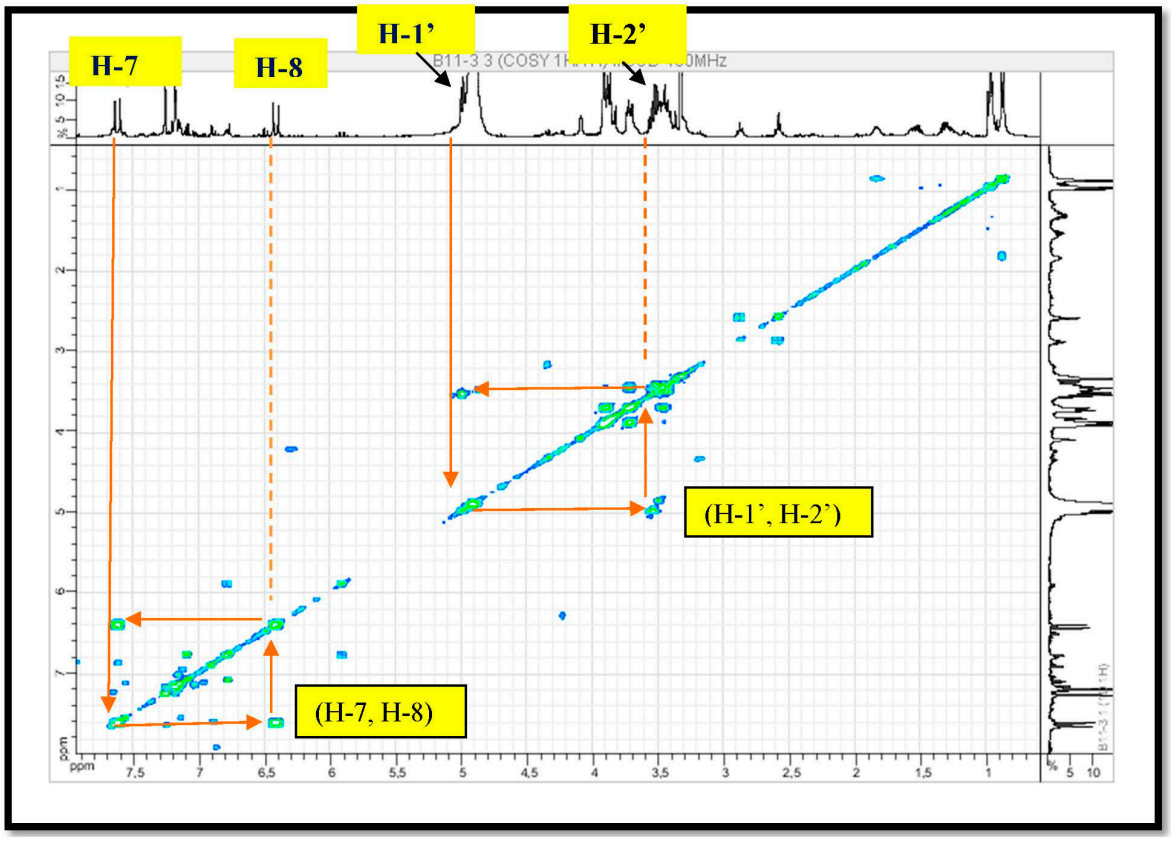


Figure S55. COSY NMR spectrum (400 MHz, CD_3OD, δppm) of lavandoside.

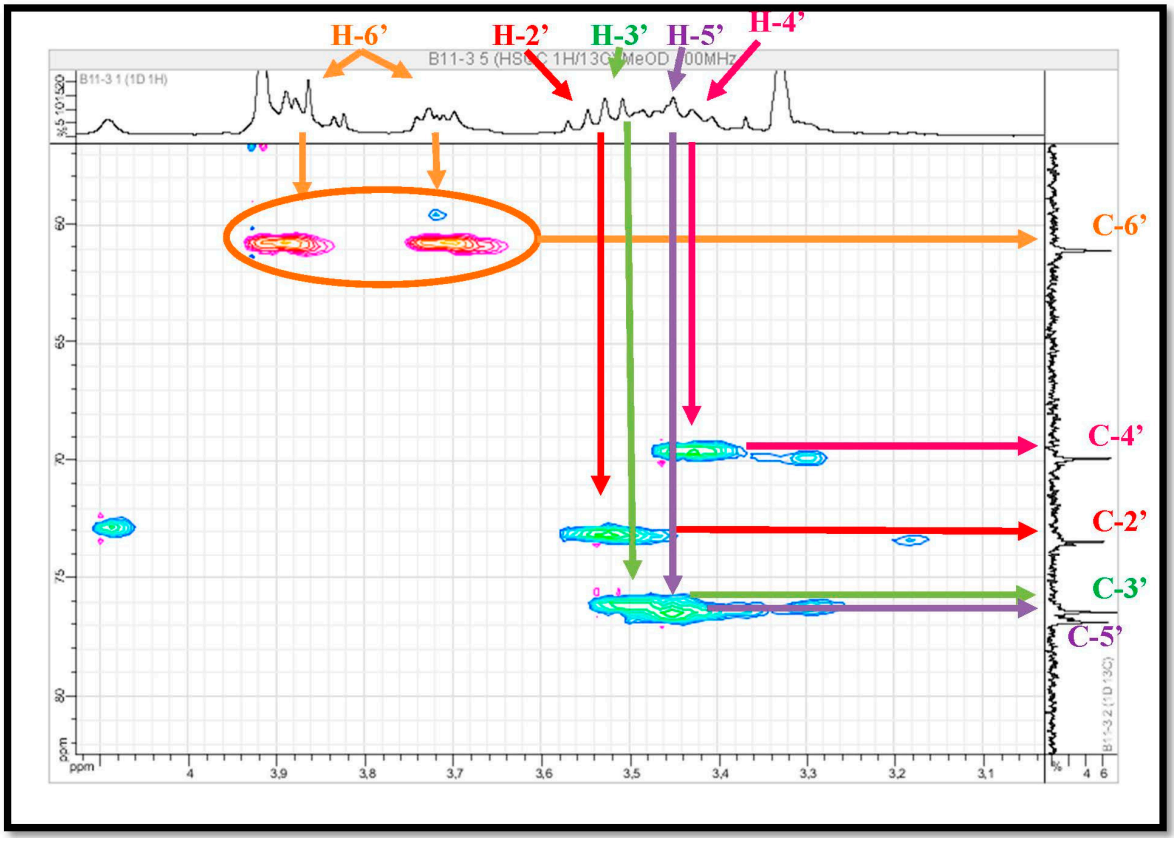


Figure S56. HSQC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of lavandoside.

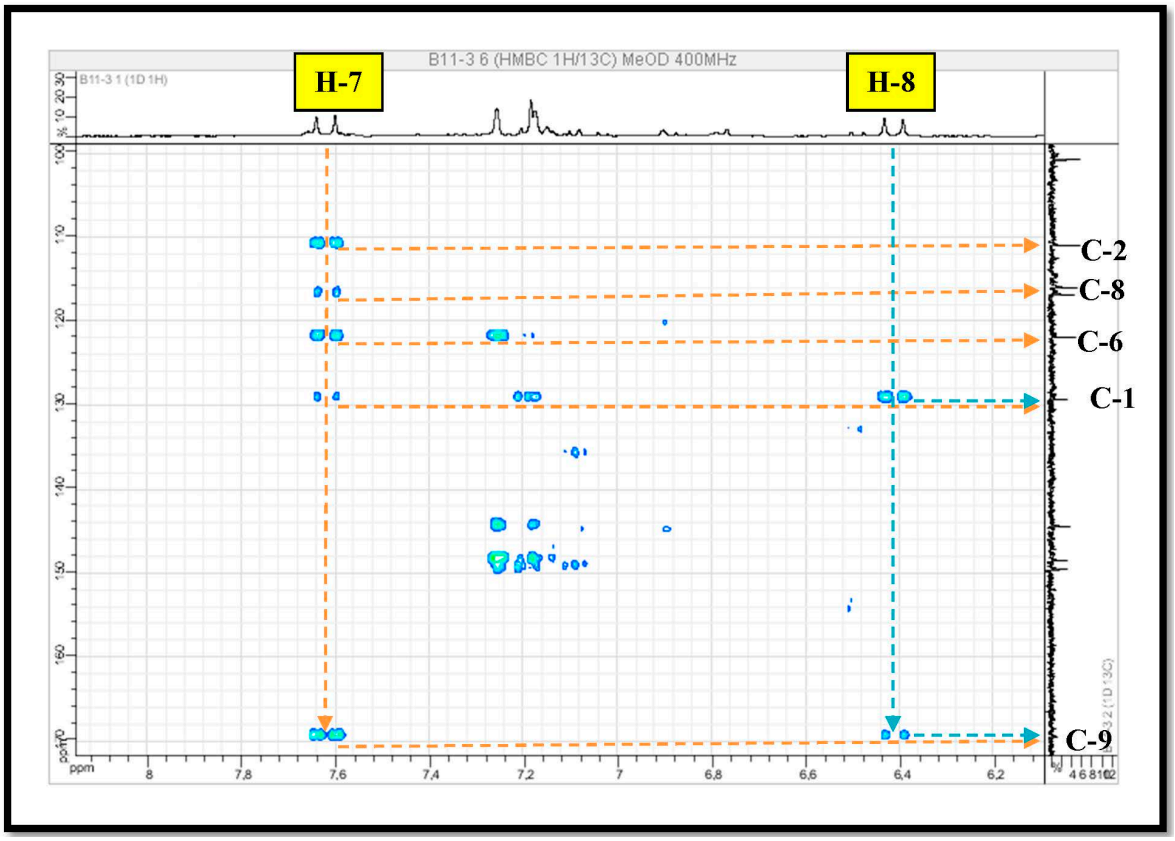


Figure S57. HMBC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of lavandoside.

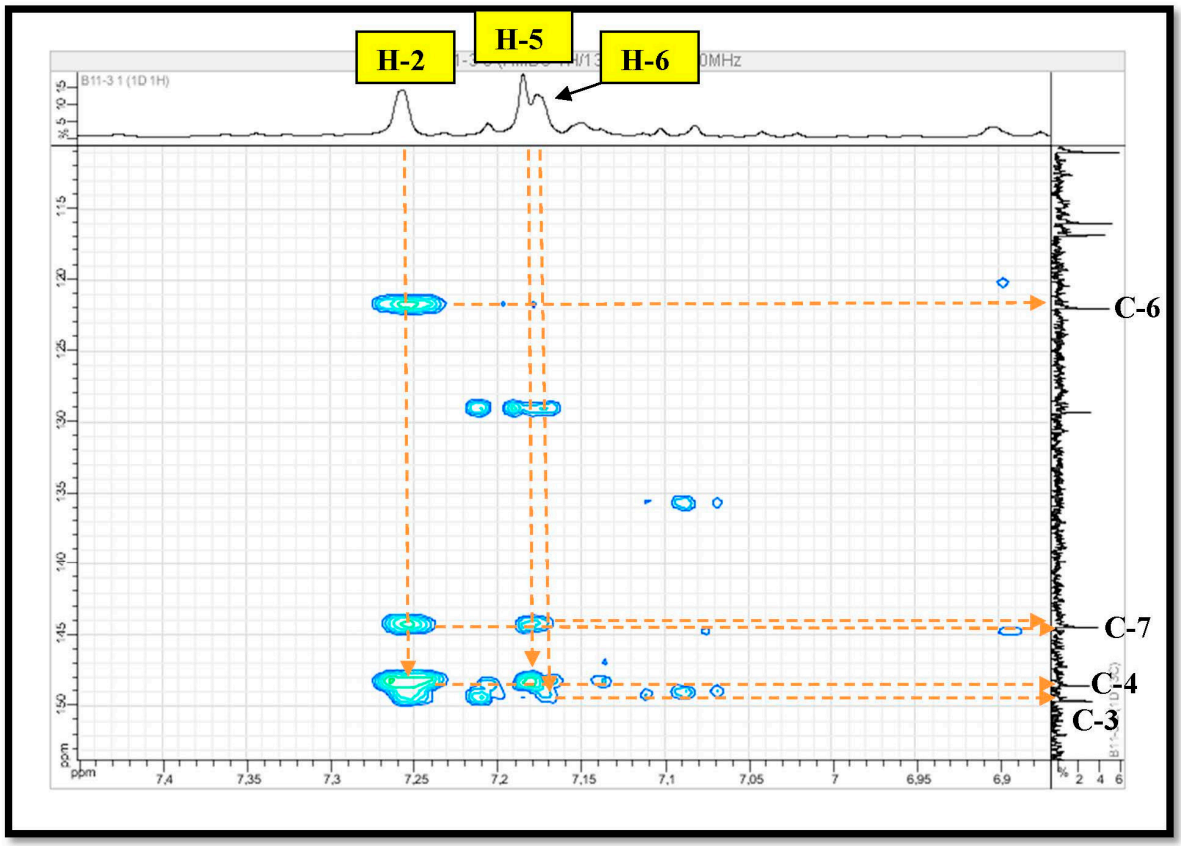


Figure S58. HMBC NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of lavandoside.

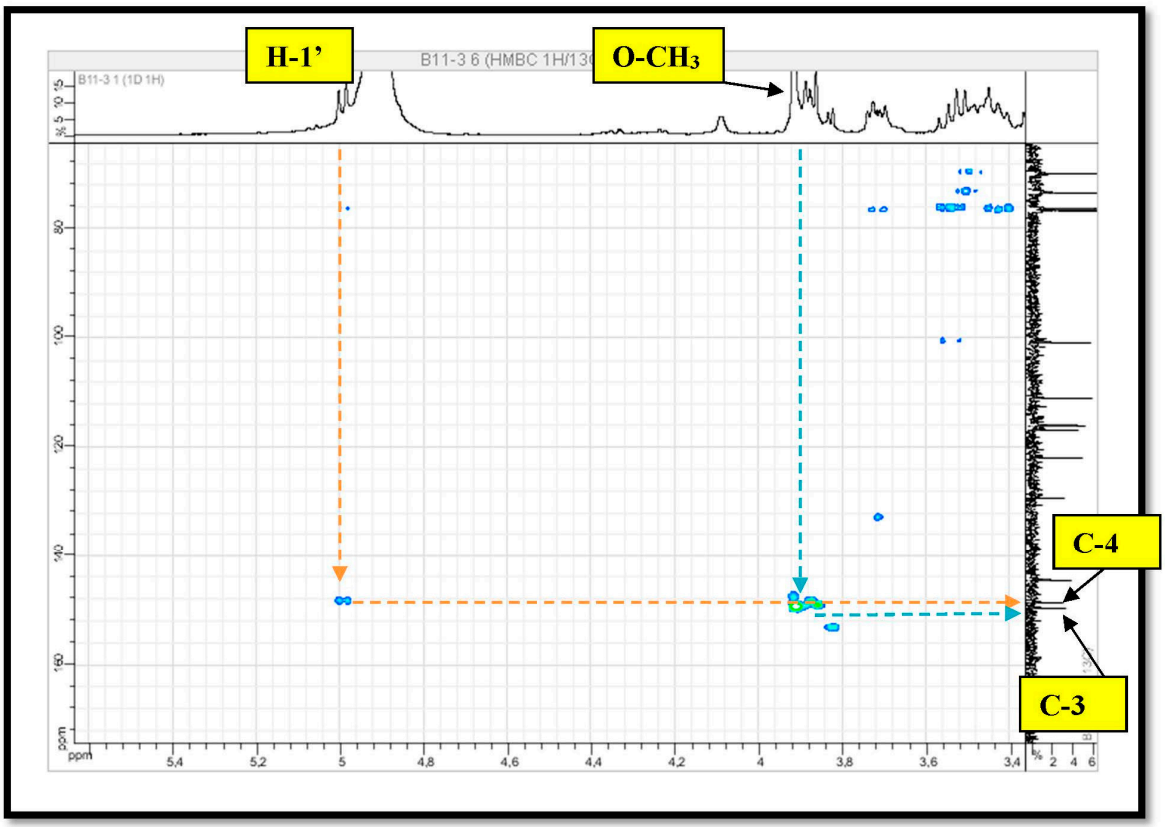


Figure S59. HMBC NMR spectrum (spreading out 3) (400 MHz, CD_3OD, δppm) of lavandoside.

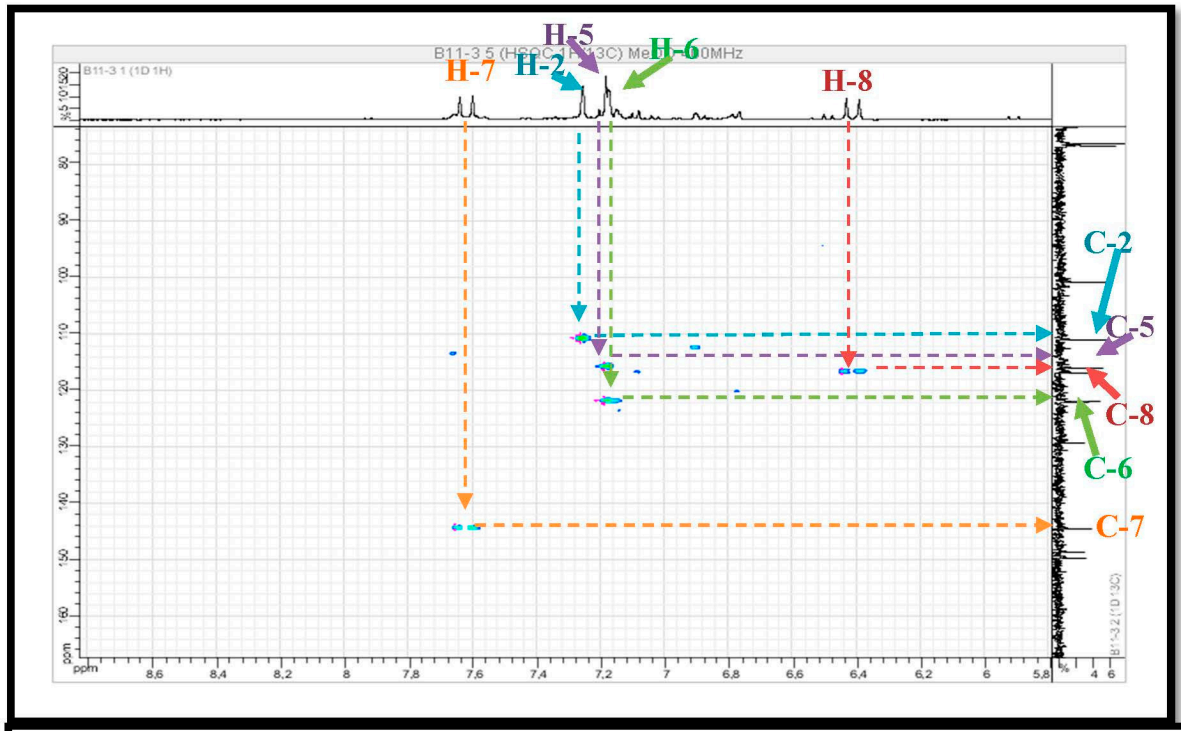


Figure S60. HSQC NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of lavandoside.

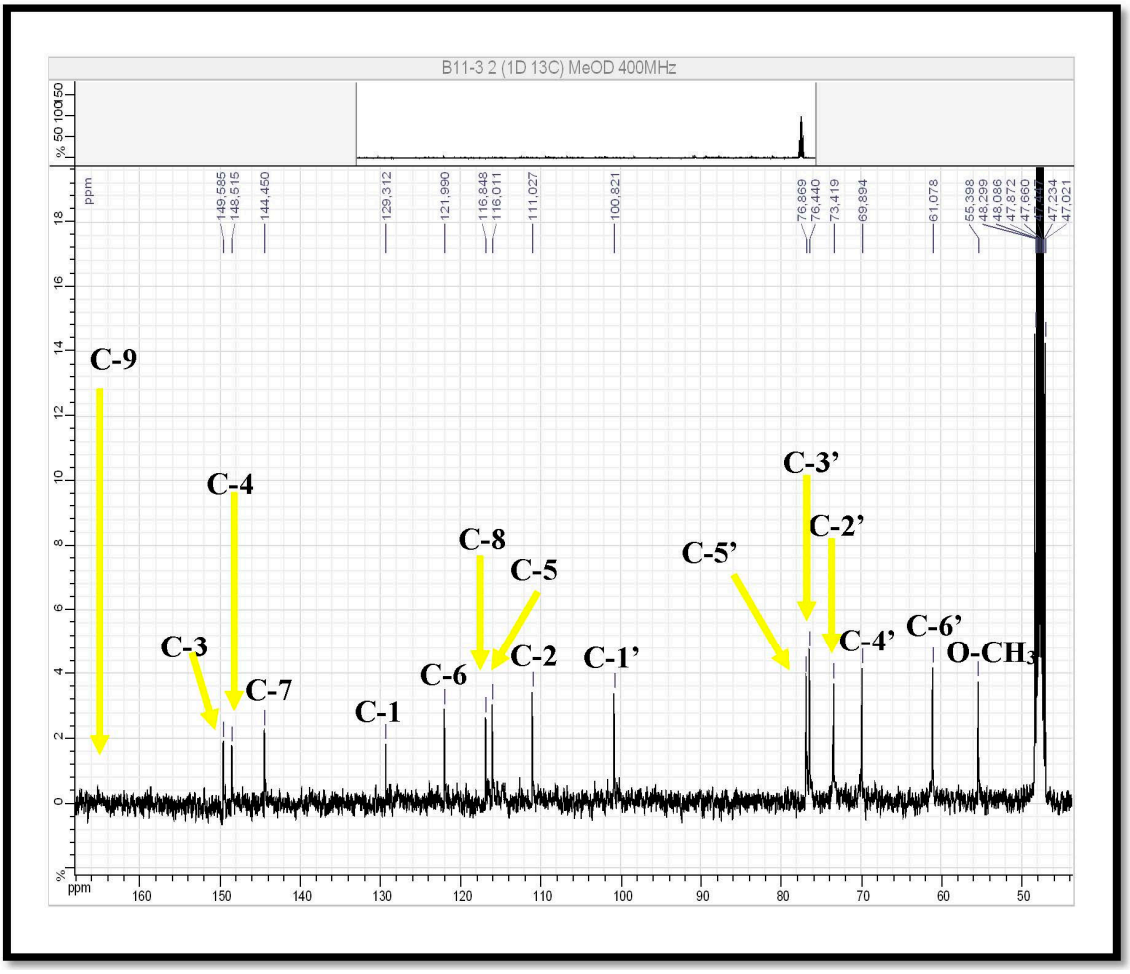
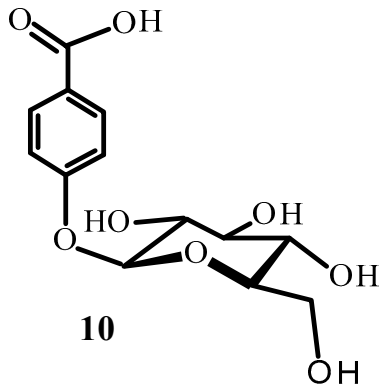
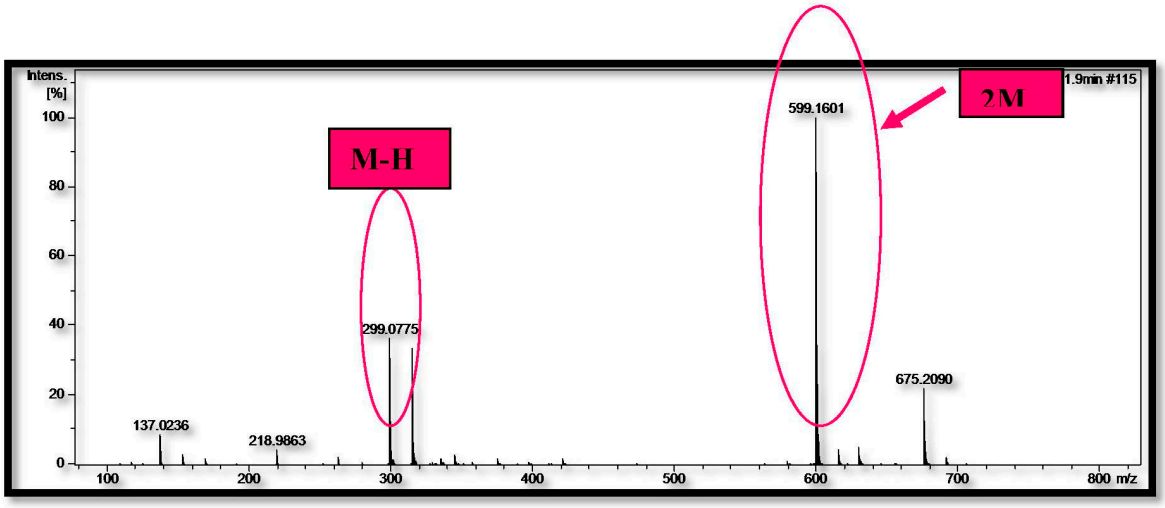


Figure S61. ^{13}C NMR spectrum (100 MHz, CD_3OD, δppm) of lavandoside.

Molecule 10: 4-Hydroxy*benzoic acid* 4-*O*-β-D-glucopyranoside



10



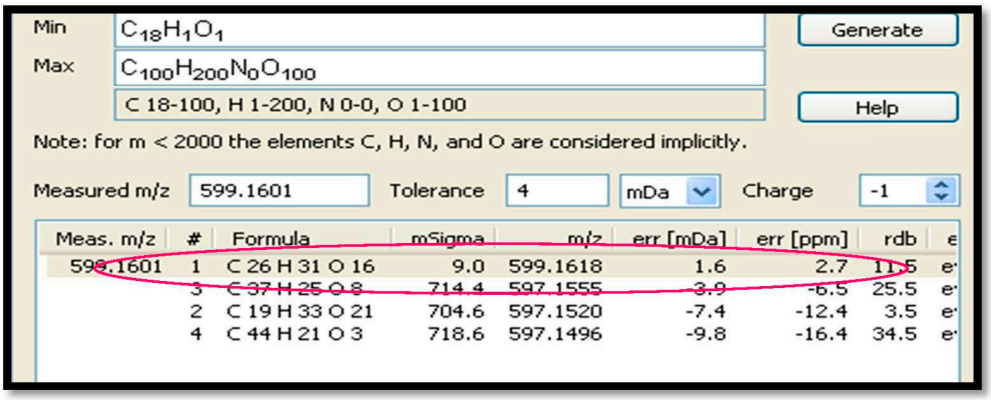


Figure S62. ESI-HRMS(−) of 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside.

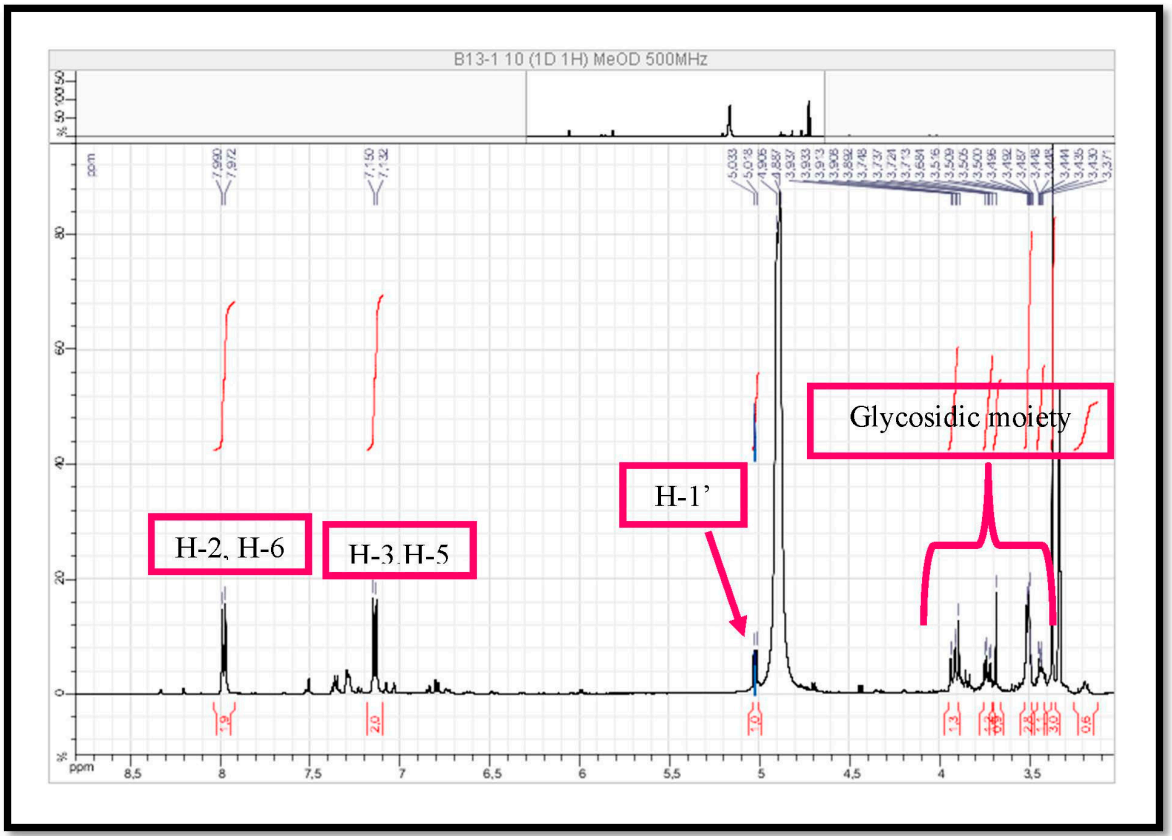


Figure S63. ^{1}H NMR spectrum (500 MHz, CD_3OD, δppm) of 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside.

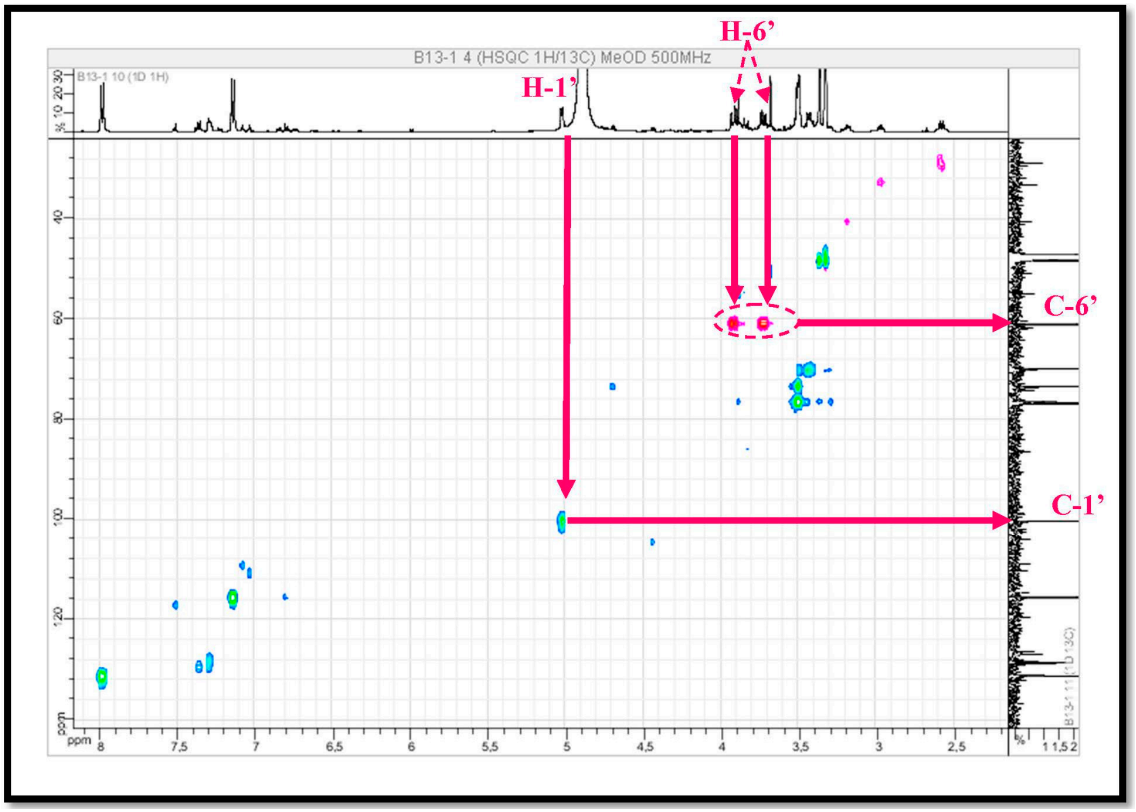


Figure S64. HSQC NMR spectrum (500 MHz, CD3OD, δppm) of 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside.

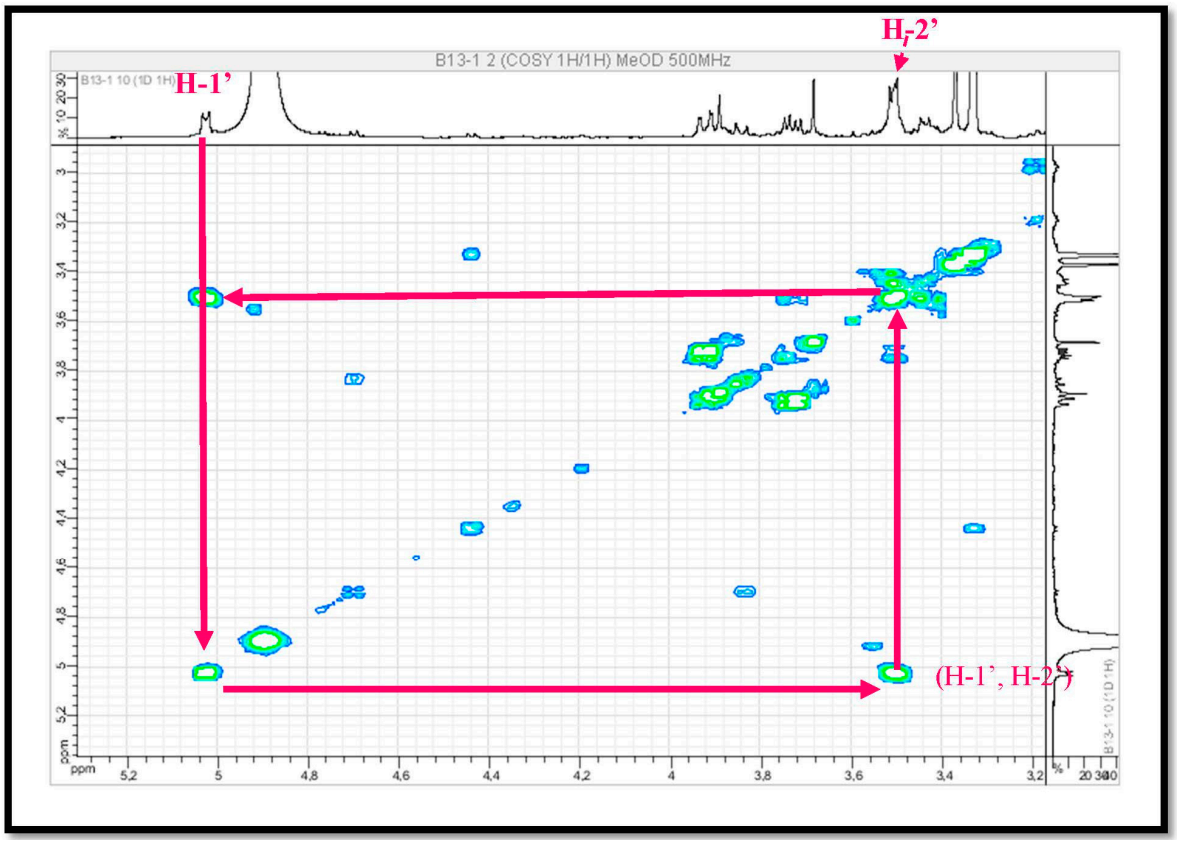


Figure S65. COSY NMR spectrum (spreading out 1) (500 MHz, CD_3OD, δppm) of 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside.

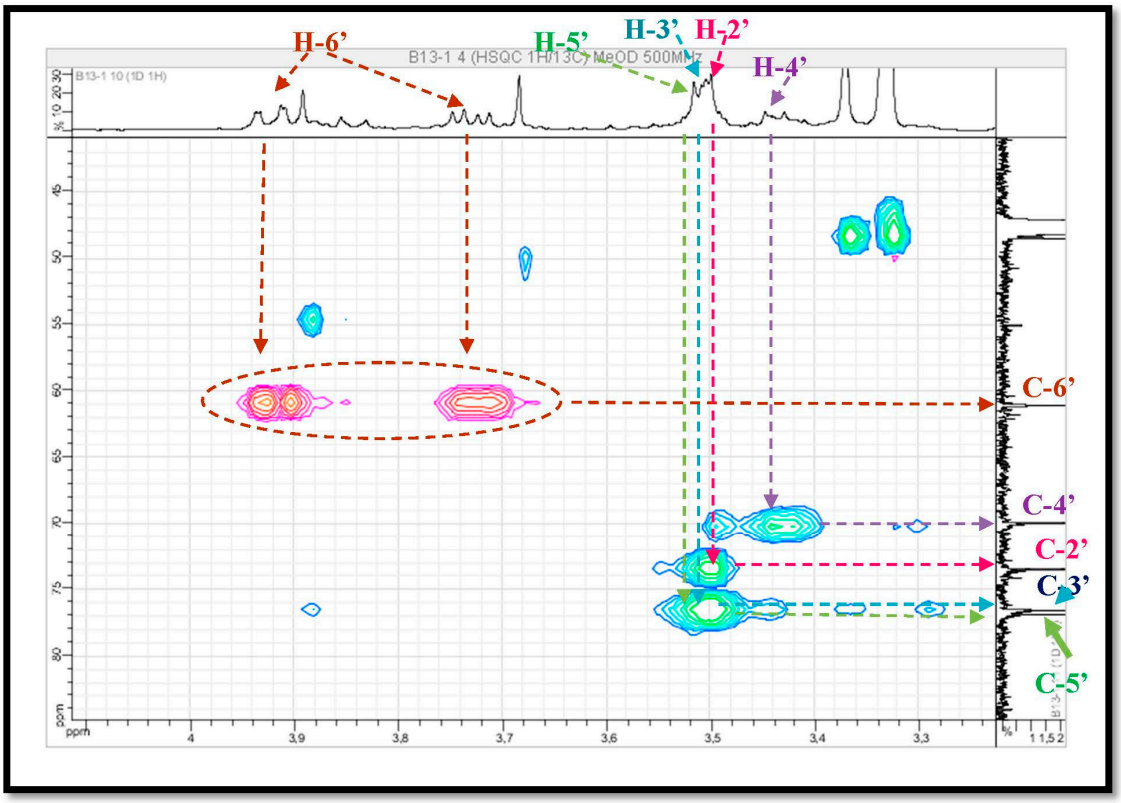


Figure S66. HSQC NMR spectrum (spreading out 1) (500 MHz, CD_3OD, δppm) of 4-hydroxybenzoic acid 4-O-β-D-glucopyranoside.

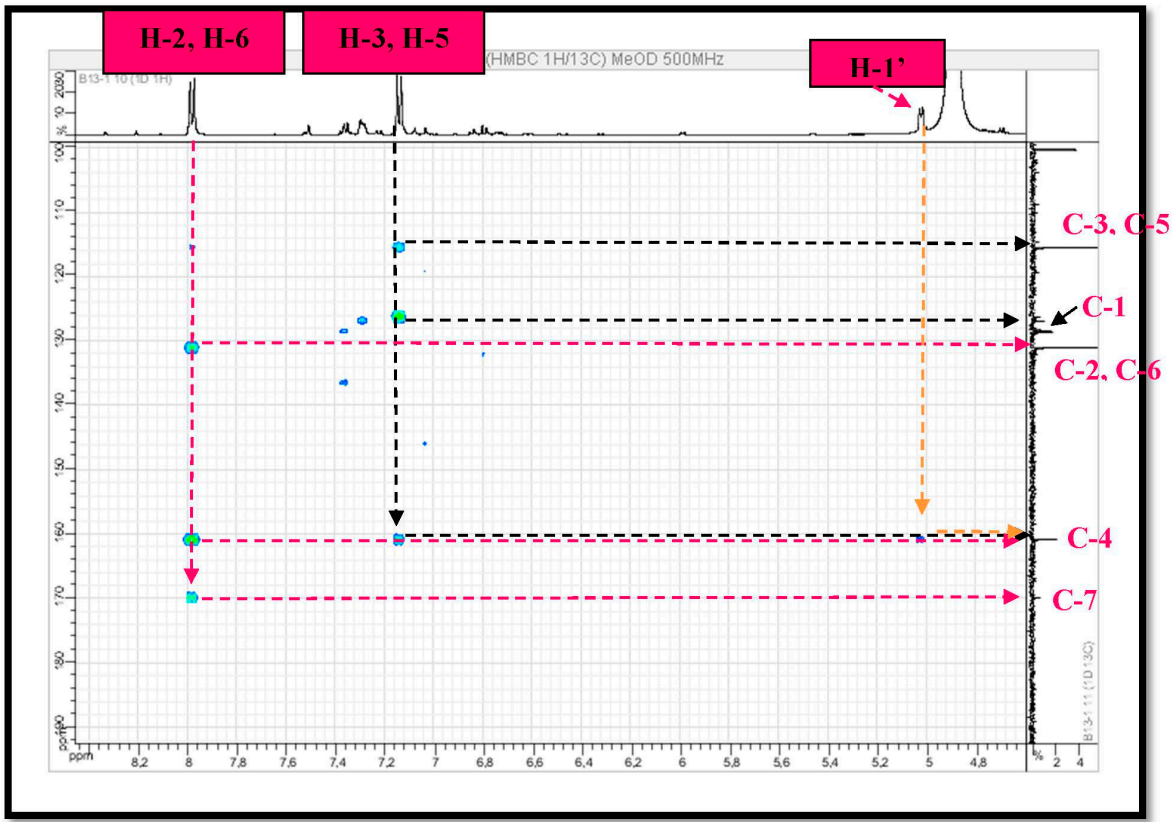


Figure S67. HMBCNMR spectrum (spreading out 1) (500 MHz, CD_3OD, δppm) of 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside.

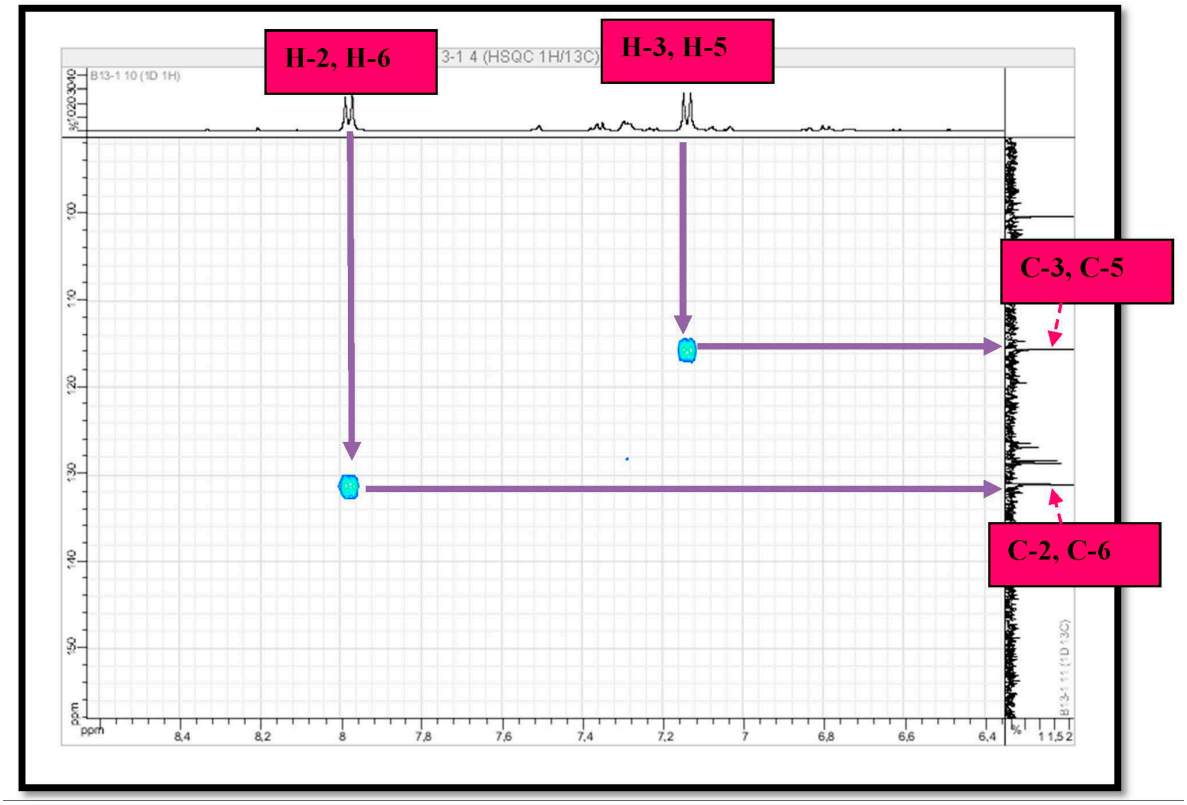


Figure S68. HSQC NMR spectrum (spreading out 2) (500 MHz, CD_3OD, δppm) of 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside.

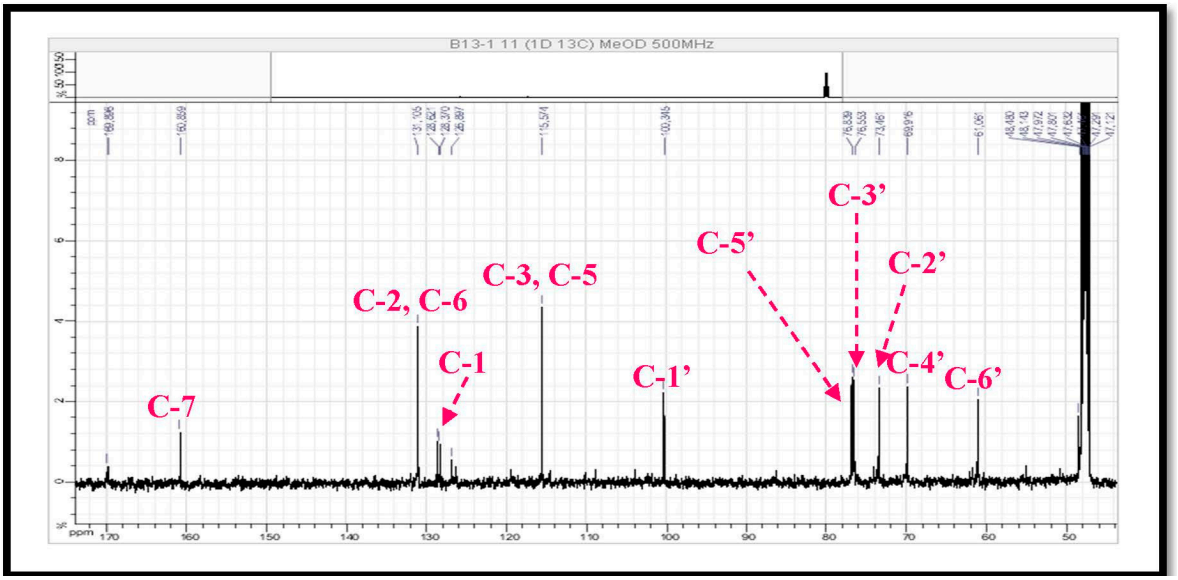
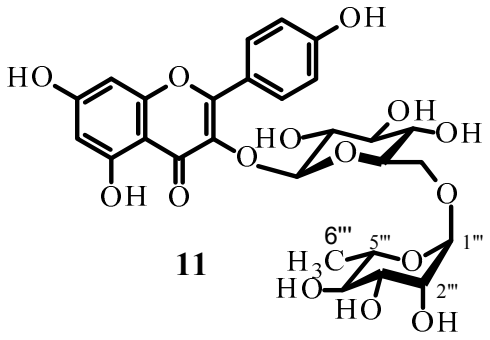


Figure S69. HSQC NMR spectrum (125 MHz, CD_3OD, δppm) of 4-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside.

Molecule 11: Nicotiflorin



11

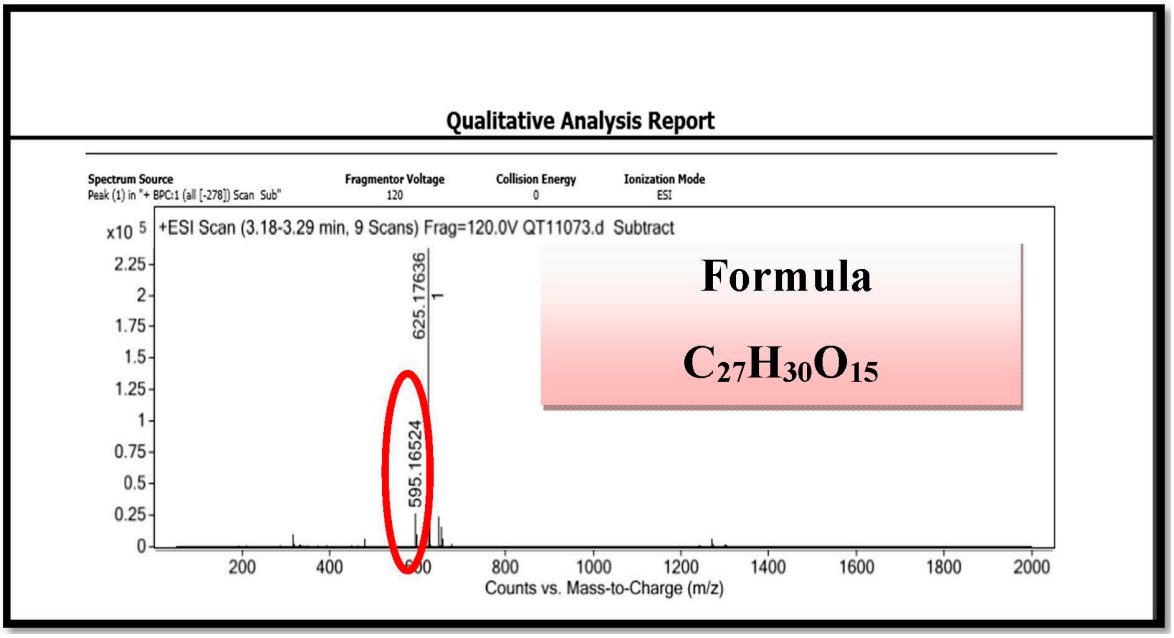


Figure S70. ESI-HRMS(+) of nicotiflorin.

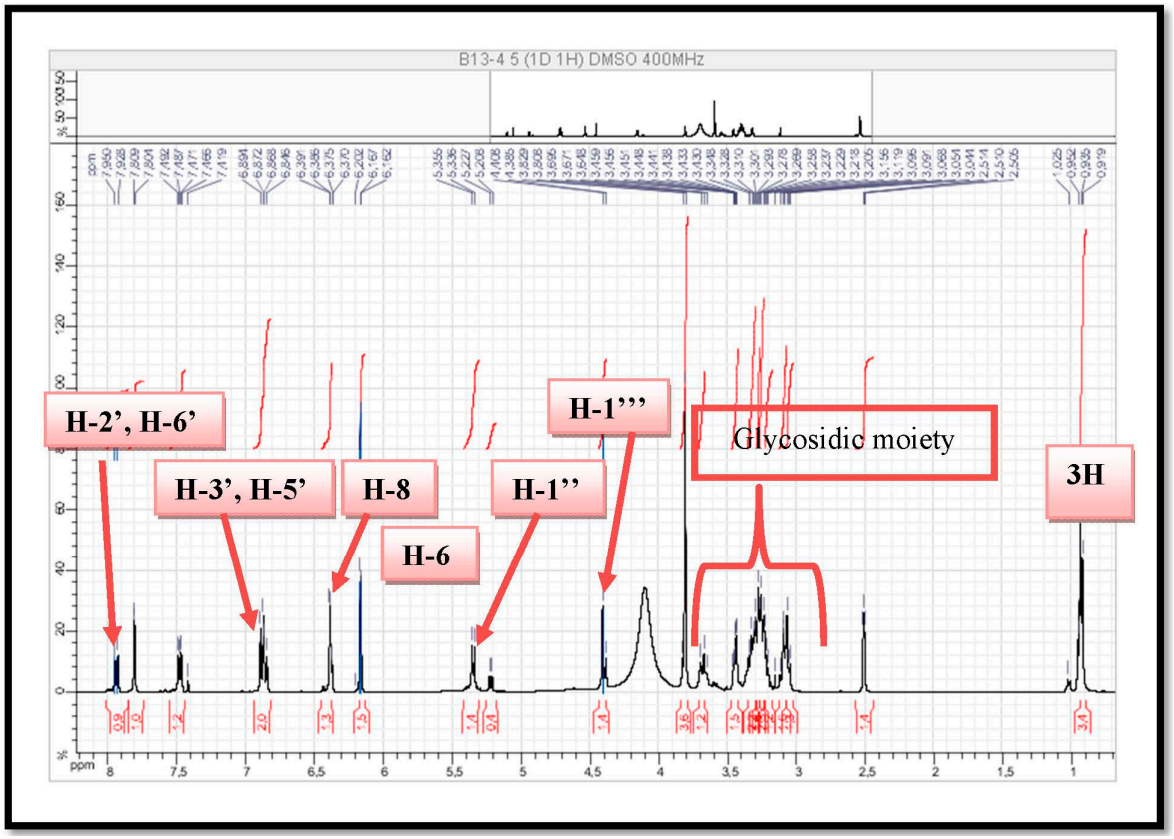


Figure S71. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

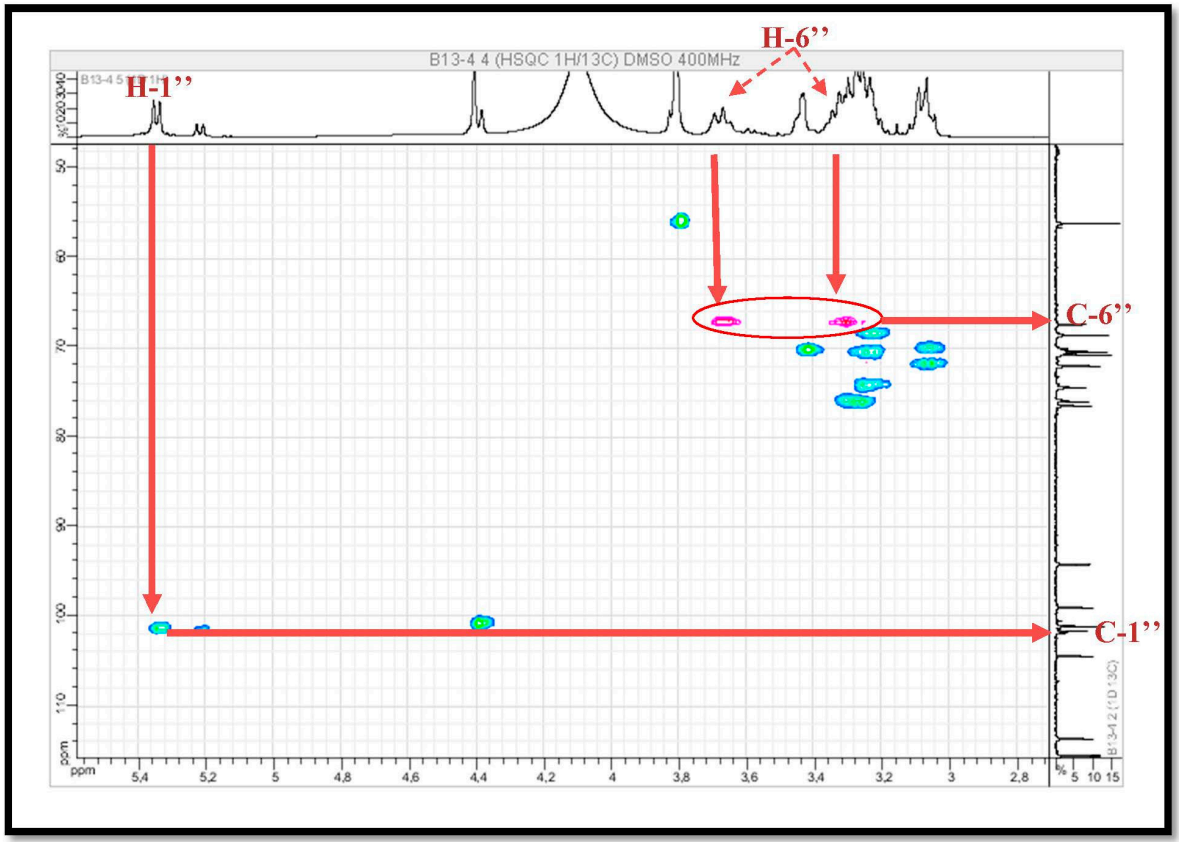


Figure S72. HSQC NMR spectrum (spreading out 1) (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

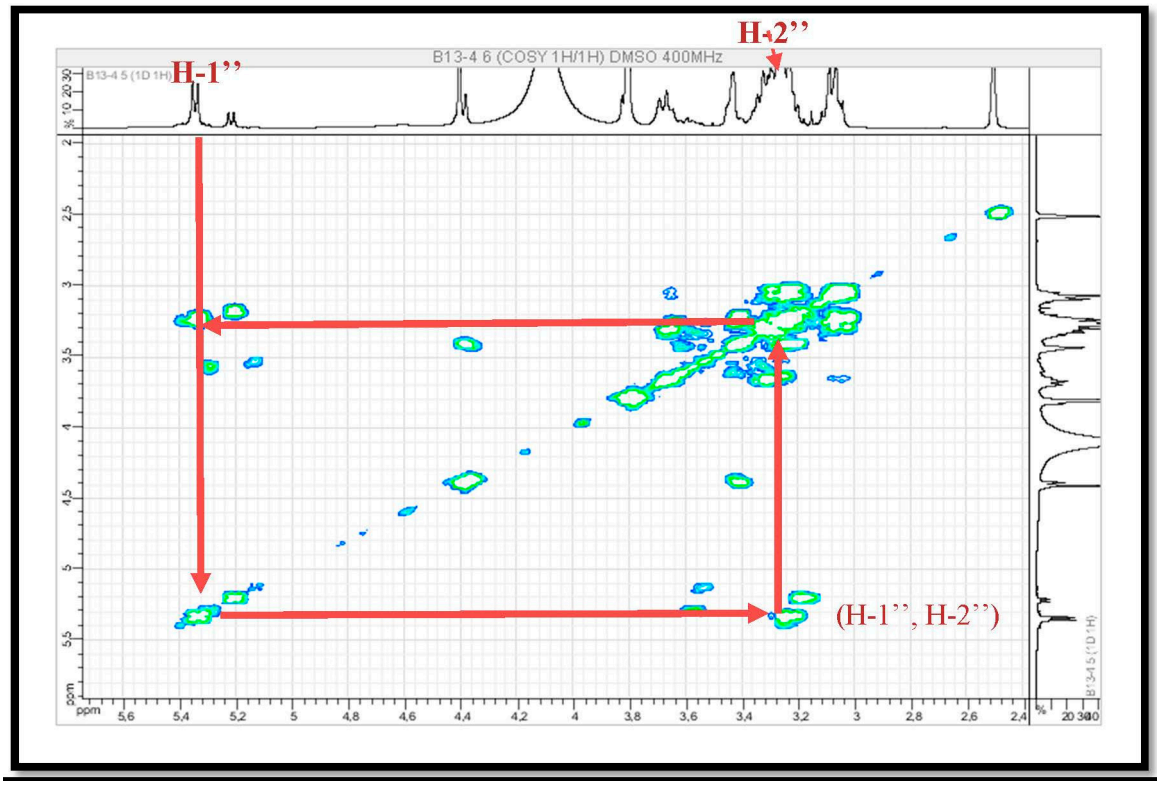


Figure S73. COSY NMR spectrum (spreading out 1) (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

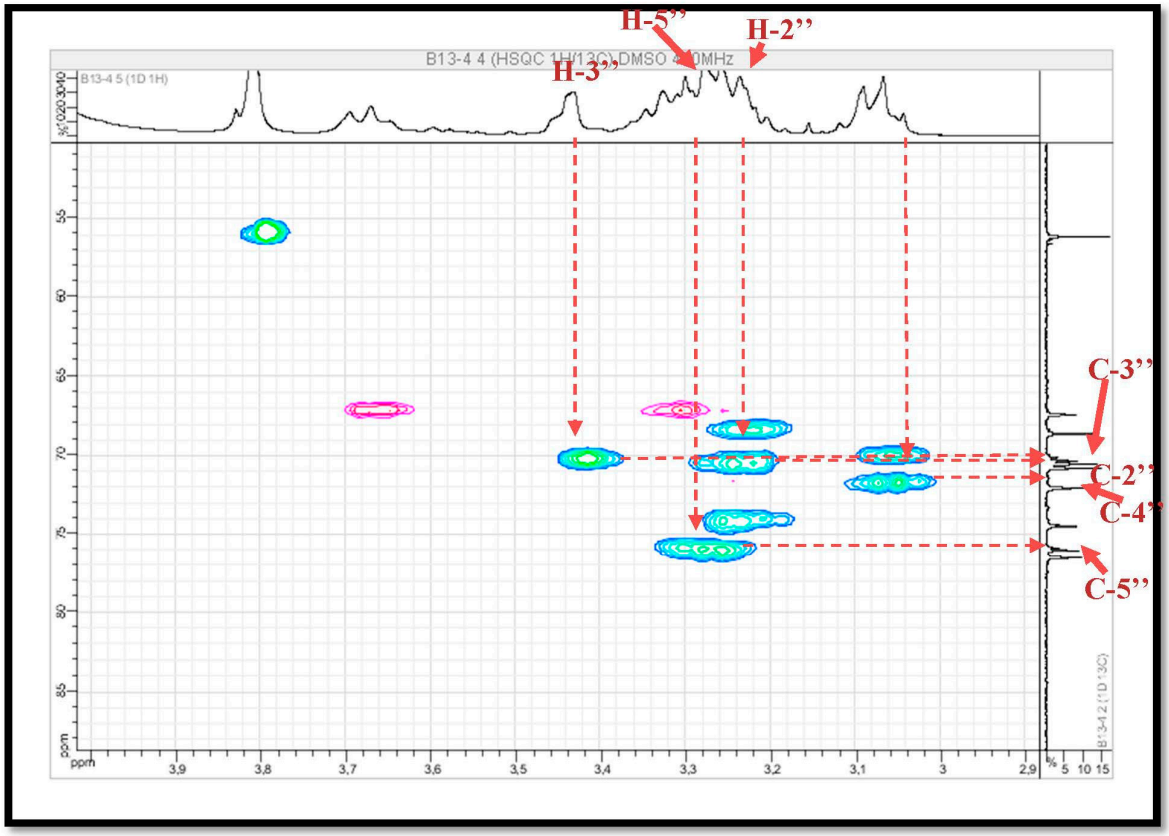


Figure S74. HSQC NMR spectrum (spreading out 2) (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

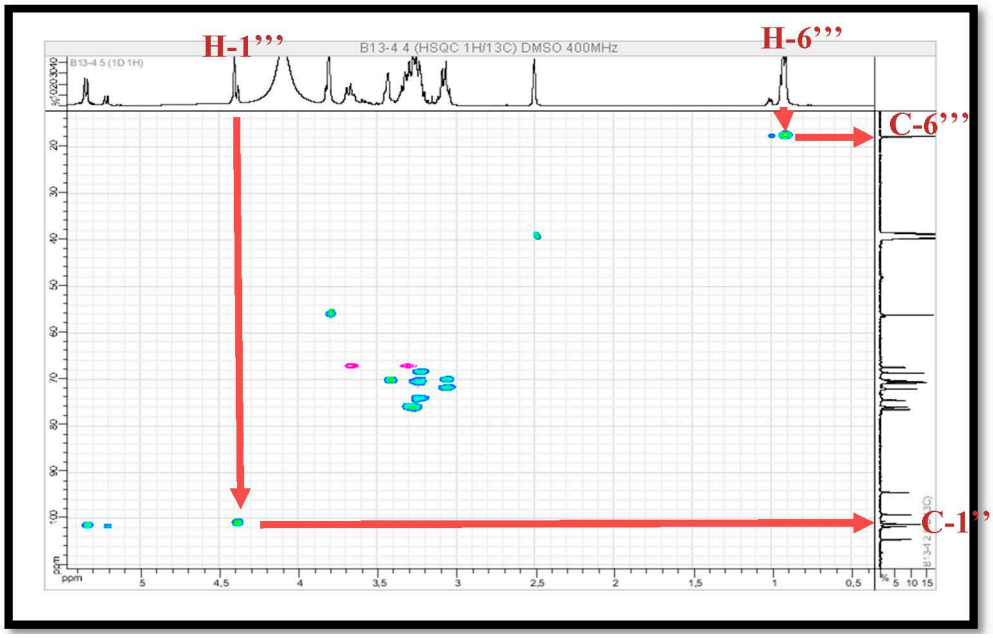


Figure S75. HSQC NMR spectrum (spreading out 3) (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

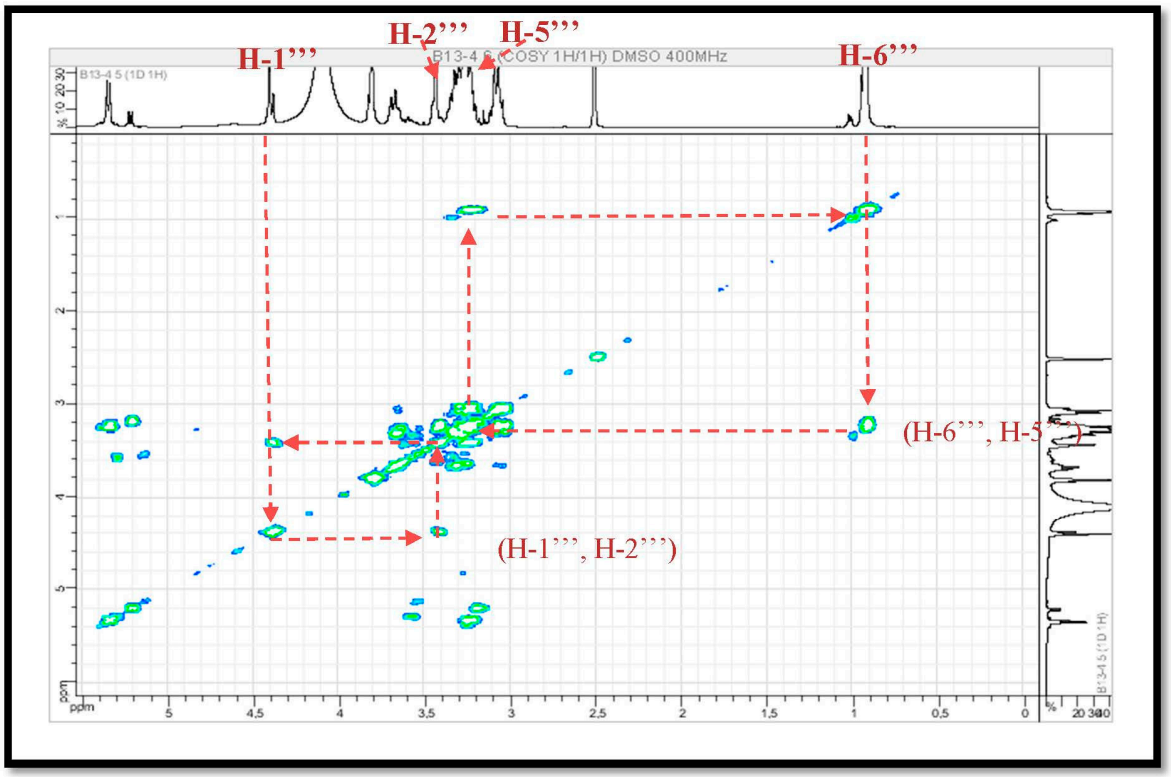


Figure S76. COSY NMR spectrum (spreading out 2) (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

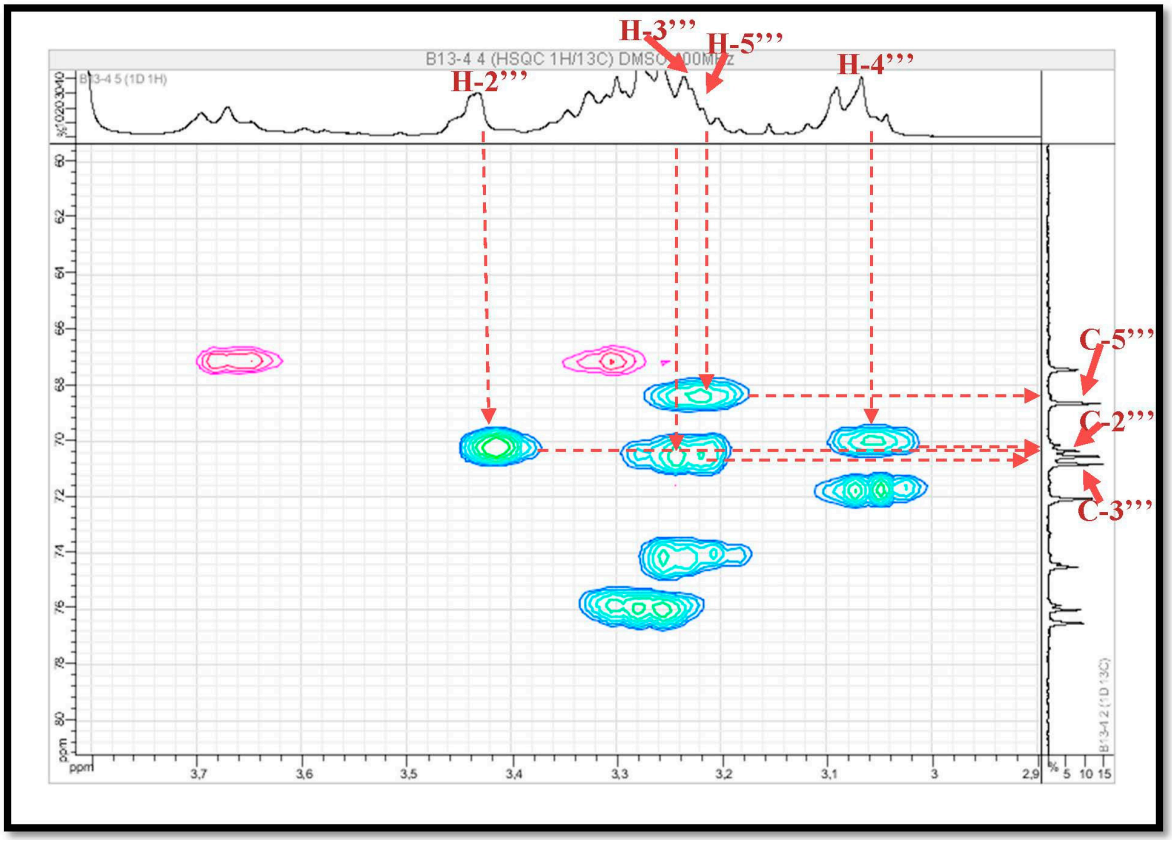


Figure S77. HSQC NMR spectrum (spreading out 4) (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

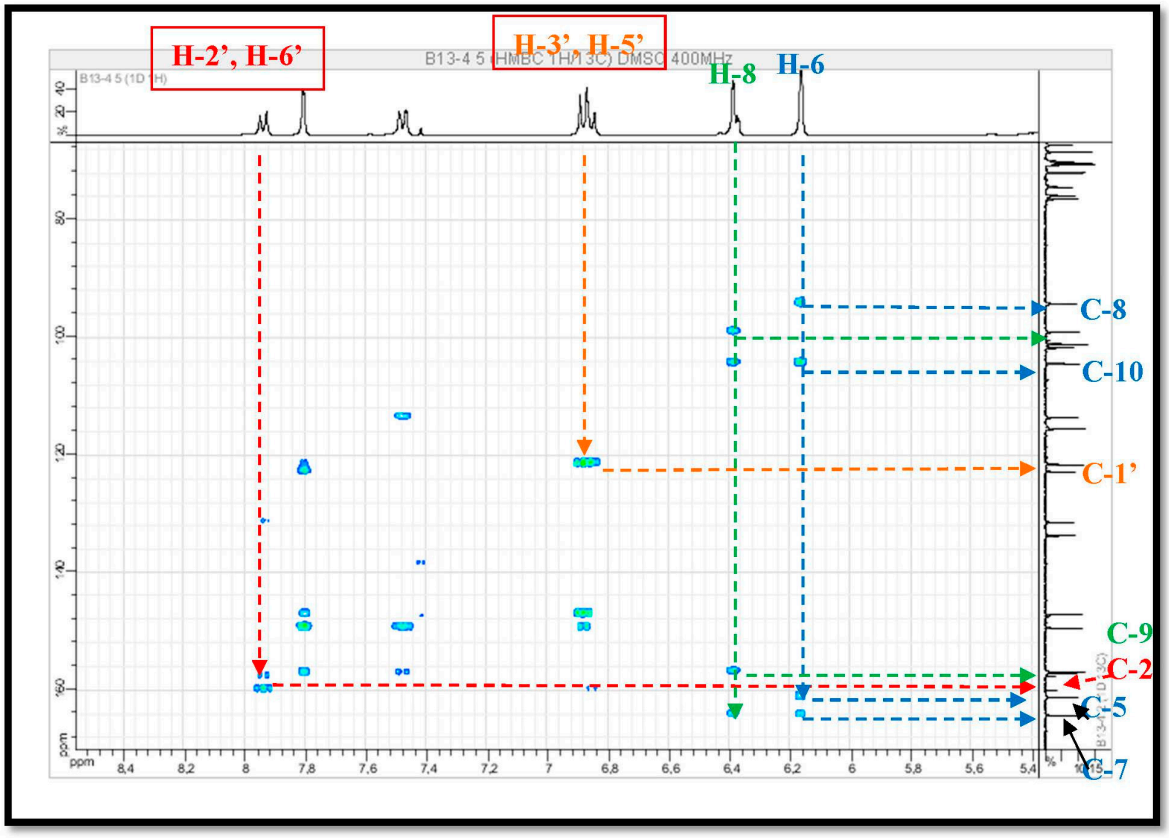


Figure S78. HMBC NMR spectrum (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

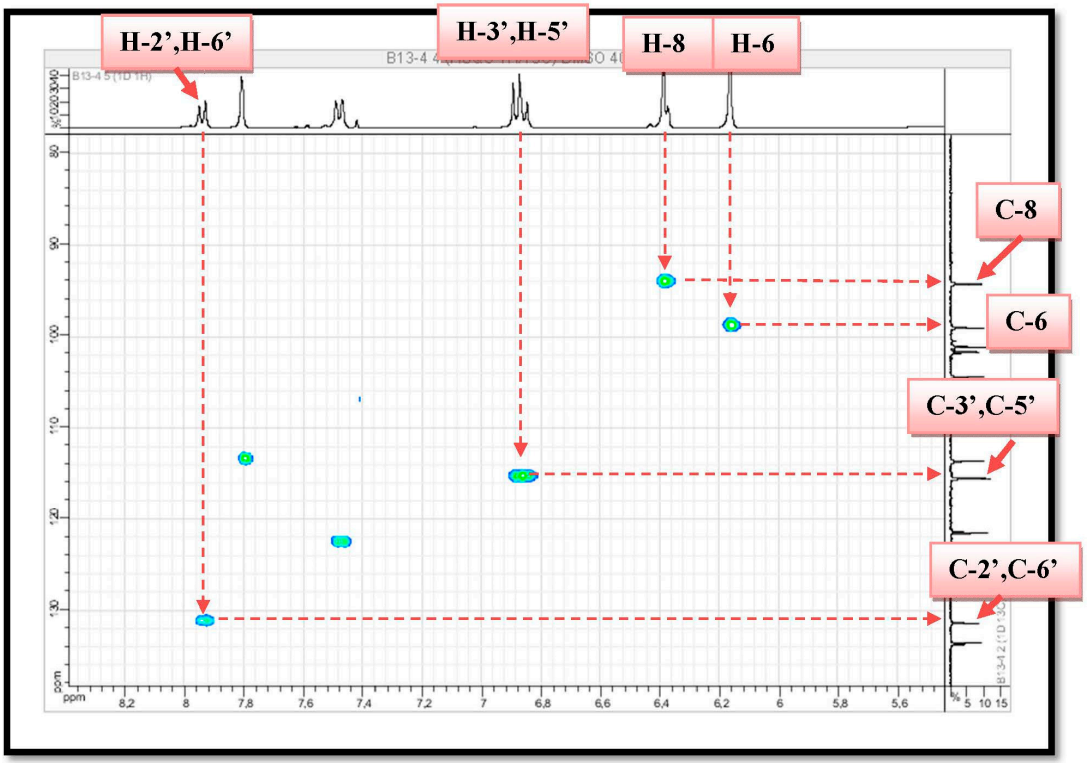


Figure S79. HSQC NMR spectrum (spreading out 5) (400 MHz, DMSO-d_6, δppm) of nicotiflorin.

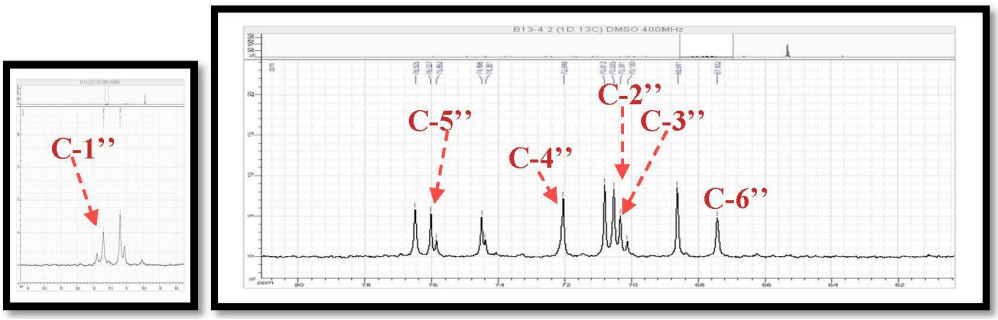


Figure S80. ^{13}C NMR spectrum (spreading out 1) (100 MHz, DMSO-d_6, δppm) of nicotiflorin.

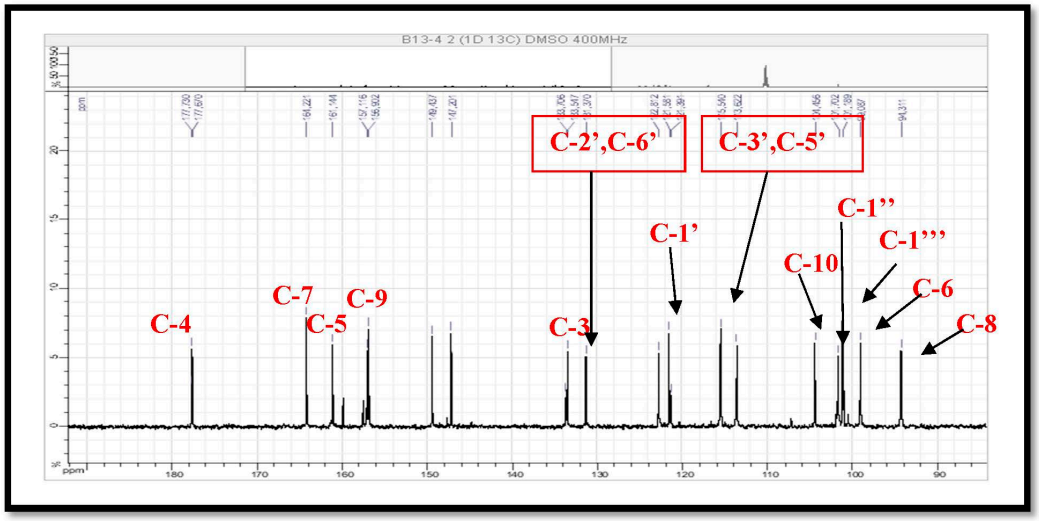
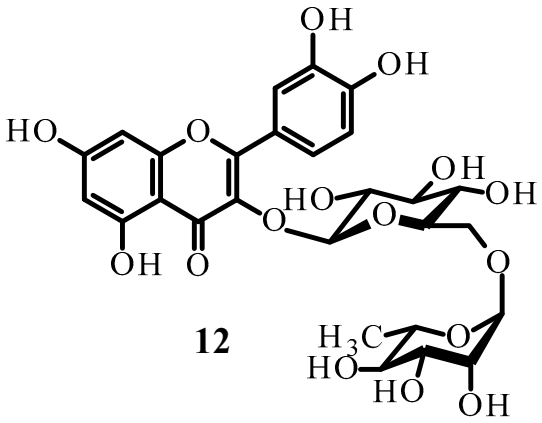
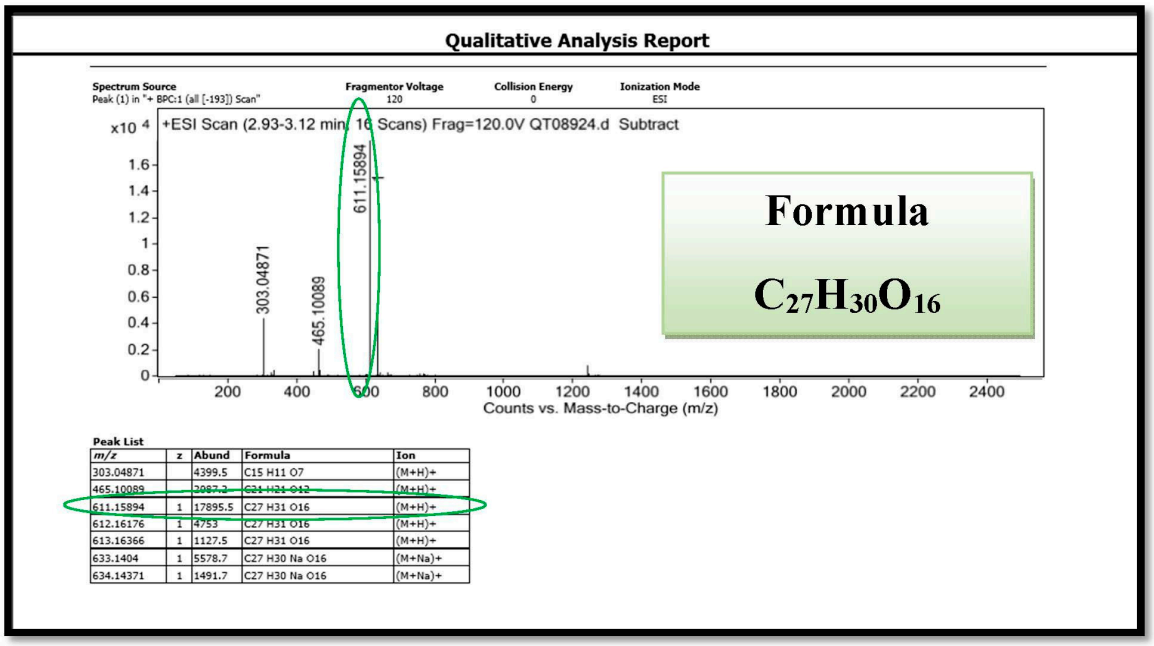


Figure S81. ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δppm) of nicotiflorin.

Molecule 12: Rutin





Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Mz	Ion Species	Score
~~C22 H30 N2 O18~~		~~610.15118~~	~~610.14936~~	~~-2.98~~	~~633.1404~~	~~C22 H30 N2 Na O18~~	95.5
C27 H30 O16	TRUE	610.15118	610.15338	3.62	633.1404	C27 H30 Na O16	91.97
~~C31 H30 O11 S~~		~~610.15118~~	~~610.15088~~	~~-0.49~~	~~633.1404~~	~~C31 H30 Na O11 S~~	88.51
C19 H34 N2 O18 S		610.15119	610.15273	2.54	633.1404	C19 H34 N2 Na O18 S	87.51
C32 H26 N4 O7 S		610.15119	610.15222	1.69	633.1404	C32 H26 N4 Na O7 S	84.18
C23 H34 N2 O13 S2		610.15119	610.15023	-1.57	633.1404	C23 H34 N2 Na O13 S2	82.51
C27 H30 O16	TRUE	610.15166	610.15338	2.83	611.15894	C27 H31 O16	93.54
C22 H30 N2 O18		610.15166	610.14936	-3.76	611.15894	C22 H31 N2 O18	92.68
C19 H34 N2 O18 S		610.15166	610.15273	1.76	611.15894	C19 H35 N2 O18 S	88.65
C31 H30 O11 S		610.15166	610.15088	-1.27	611.15894	C31 H31 O11 S	87.09
C32 H26 N4 O7 S		610.15166	610.15222	0.91	611.15894	C32 H27 N4 O7 S	84.82
C23 H34 N2 O13 S2		610.15166	610.15023	-2.35	611.15894	C23 H35 N2 O13 S2	80.33
C21 H20 O12	TRUE	464.09361	464.09548	4.02	465.10089	C21 H21 O12	93.12
C25 H20 O7 S		464.09361	464.09297	-1.38	465.10089	C25 H21 O7 S	89.29
C16 H20 N2 O14		464.09361	464.09145	-4.66	465.10089	C16 H21 N2 O14	89.1
C26 H16 N4 O3 S		464.09362	464.09431	1.5	465.10089	C26 H17 N4 O3 S	86.05
C13 H24 N2 O14 S		464.09362	464.09482	2.6	465.10089	C13 H25 N2 O14 S	83.01
C15 H10 O7	TRUE	302.04143	302.04265	4.03	303.04871	C15 H11 O7	95.35
C19 H10 O2 S		302.04144	302.04015	-4.26	303.04871	C19 H11 O2 S	83.83
C7 H14 N2 O9 S		302.04144	302.042	1.86	303.04871	C7 H15 N2 O9 S	80.49

Figure S82. ESI-HRMS(+) of rutin.

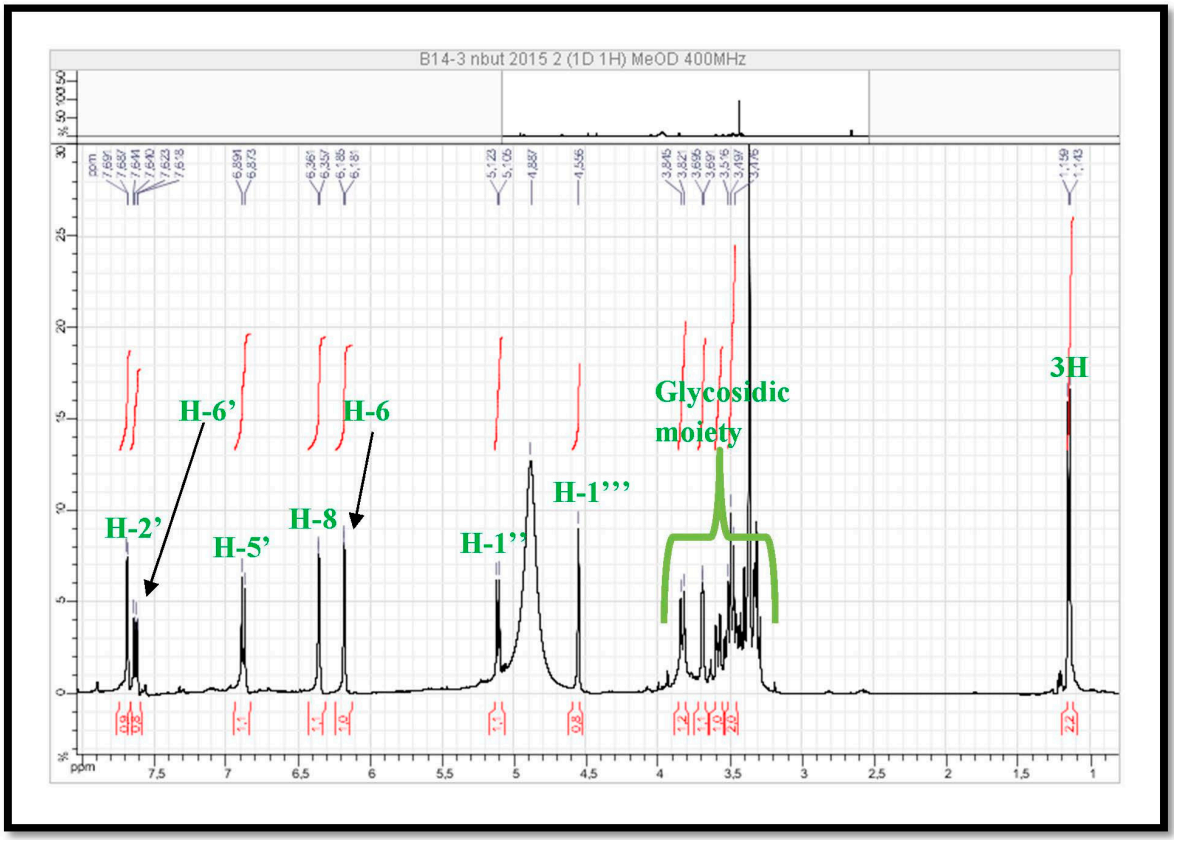


Figure S83. ^{1}H NMR spectrum (400 MHz, CD_3OD, δppm) of rutin.

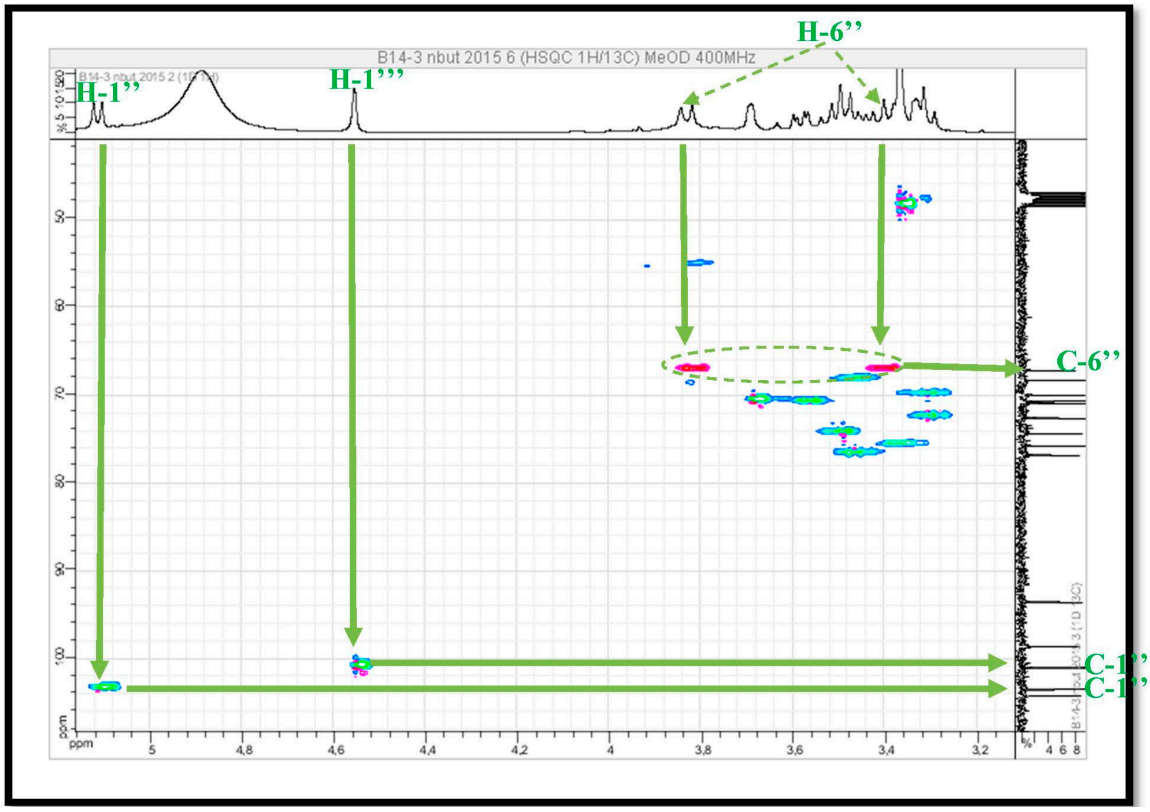


Figure S84. HSQC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of rutin.

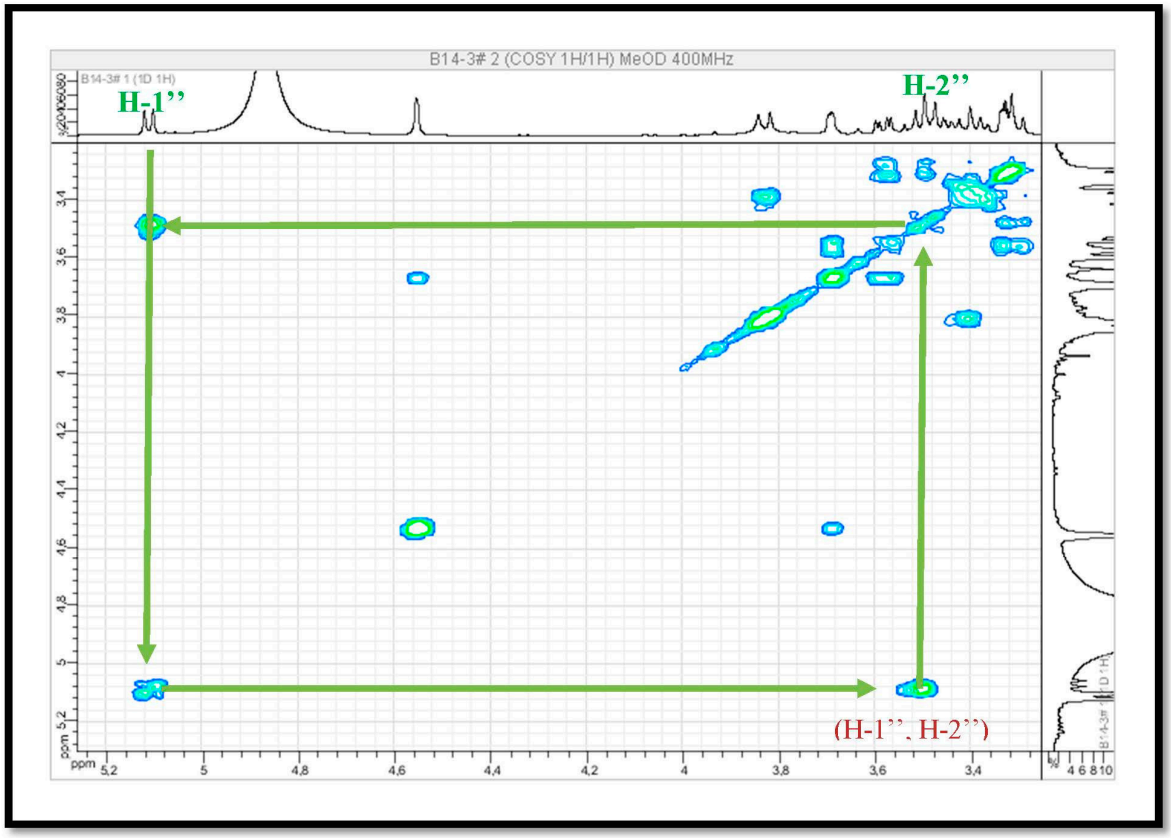


Figure S85. COSY NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of rutin.

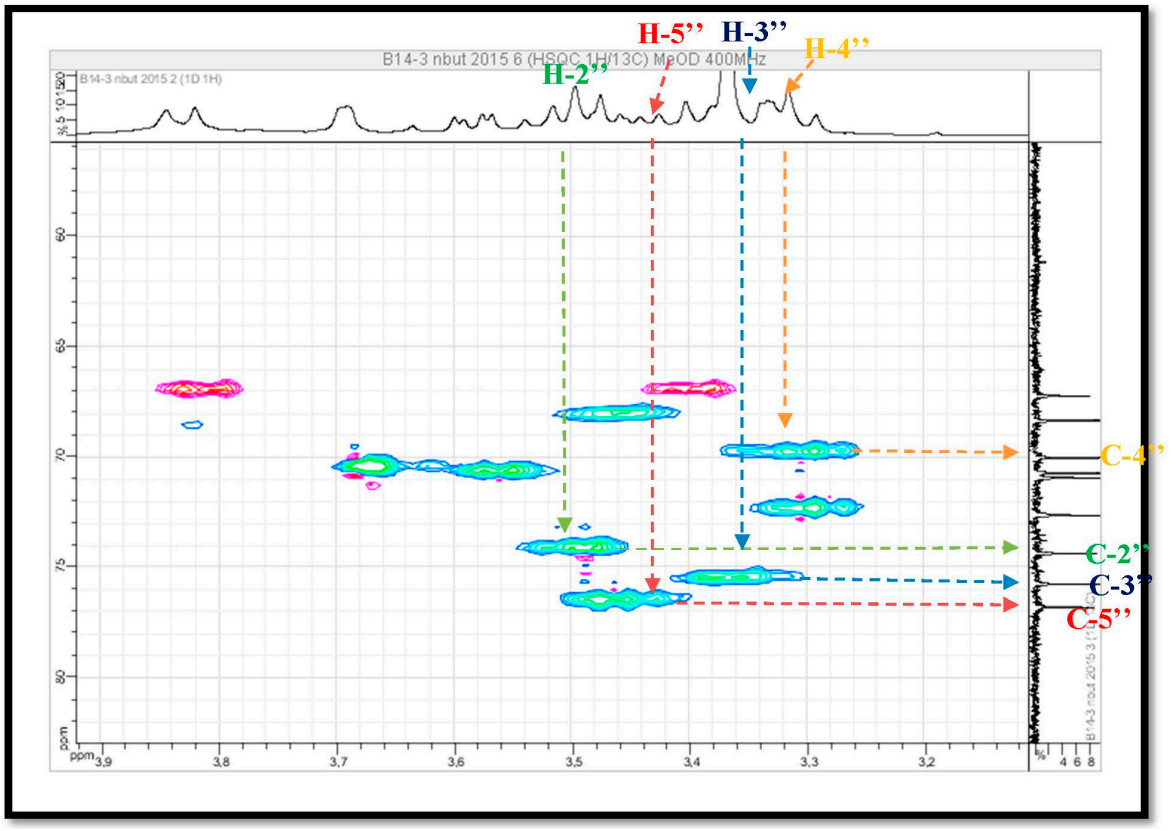


Figure S86. HSQC NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of rutin.

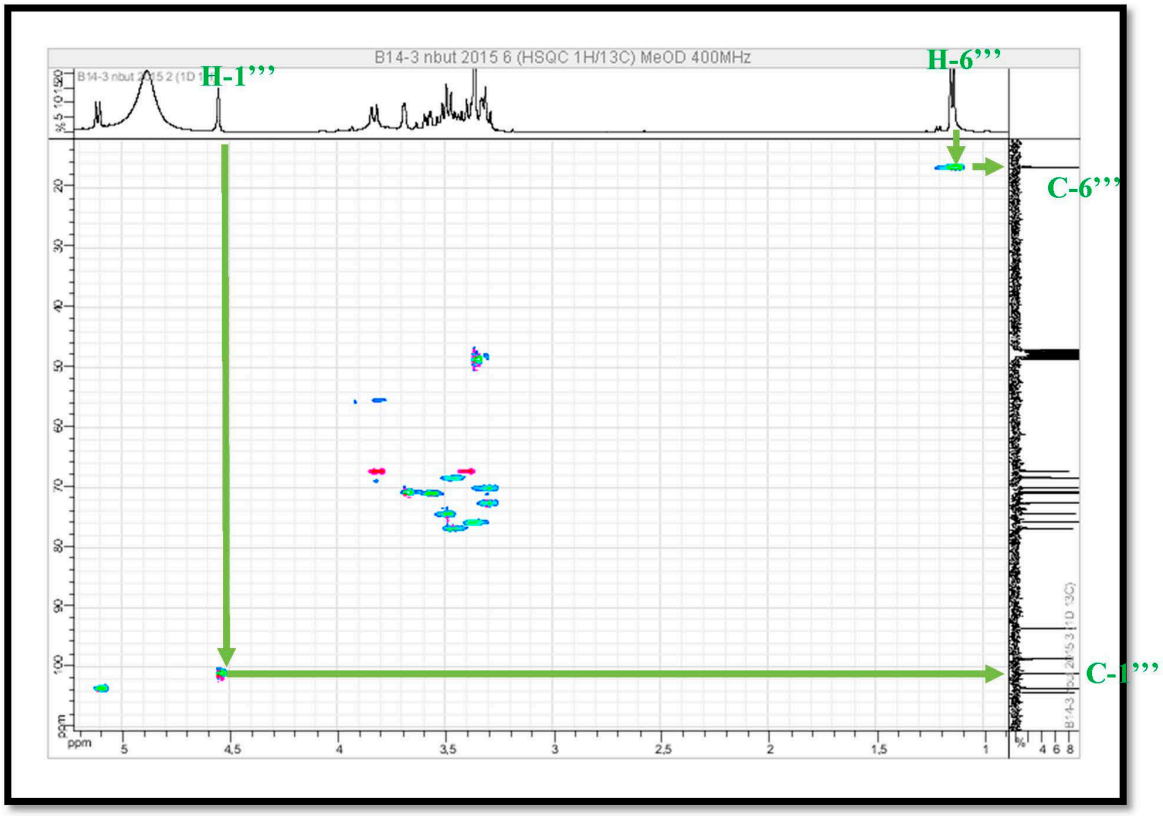


Figure S87. HSQC NMR spectrum (spreading out 3) (400 MHz, CD_3OD, δppm) of rutin.

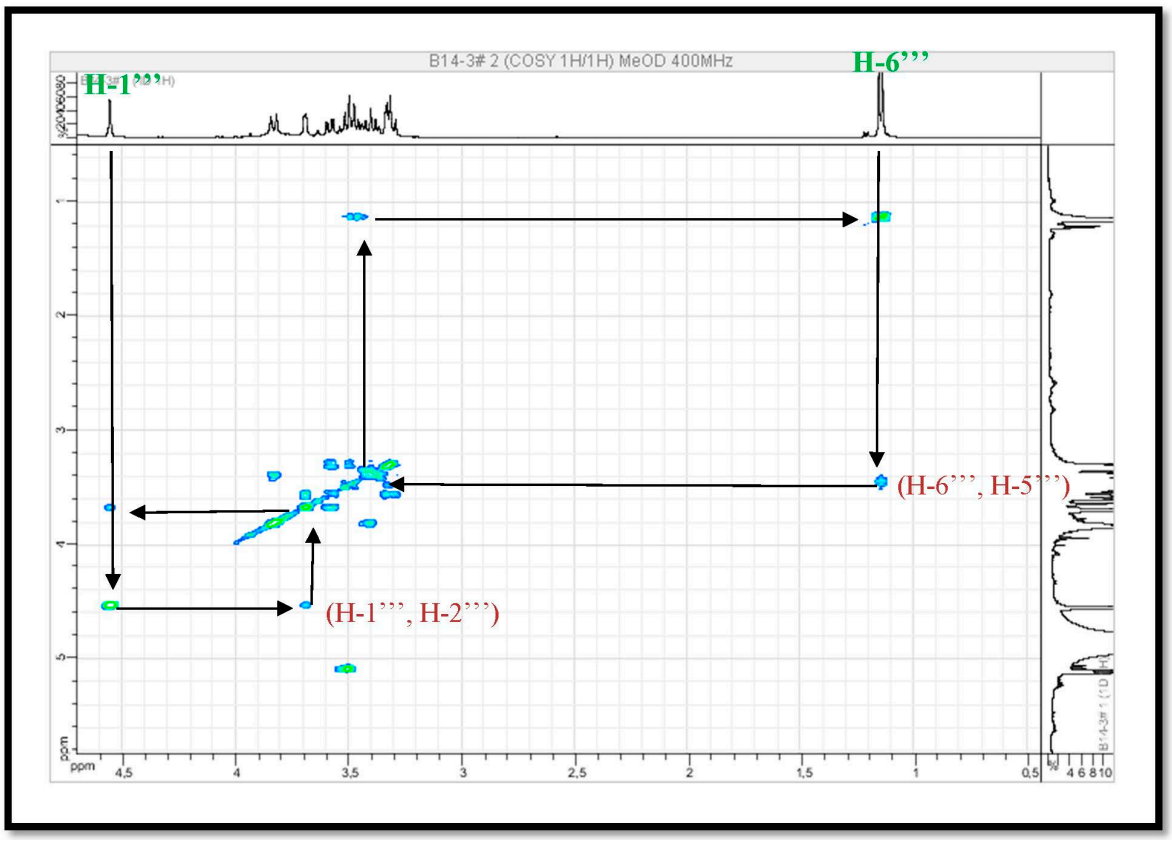


Figure S88. COSY NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of rutin.

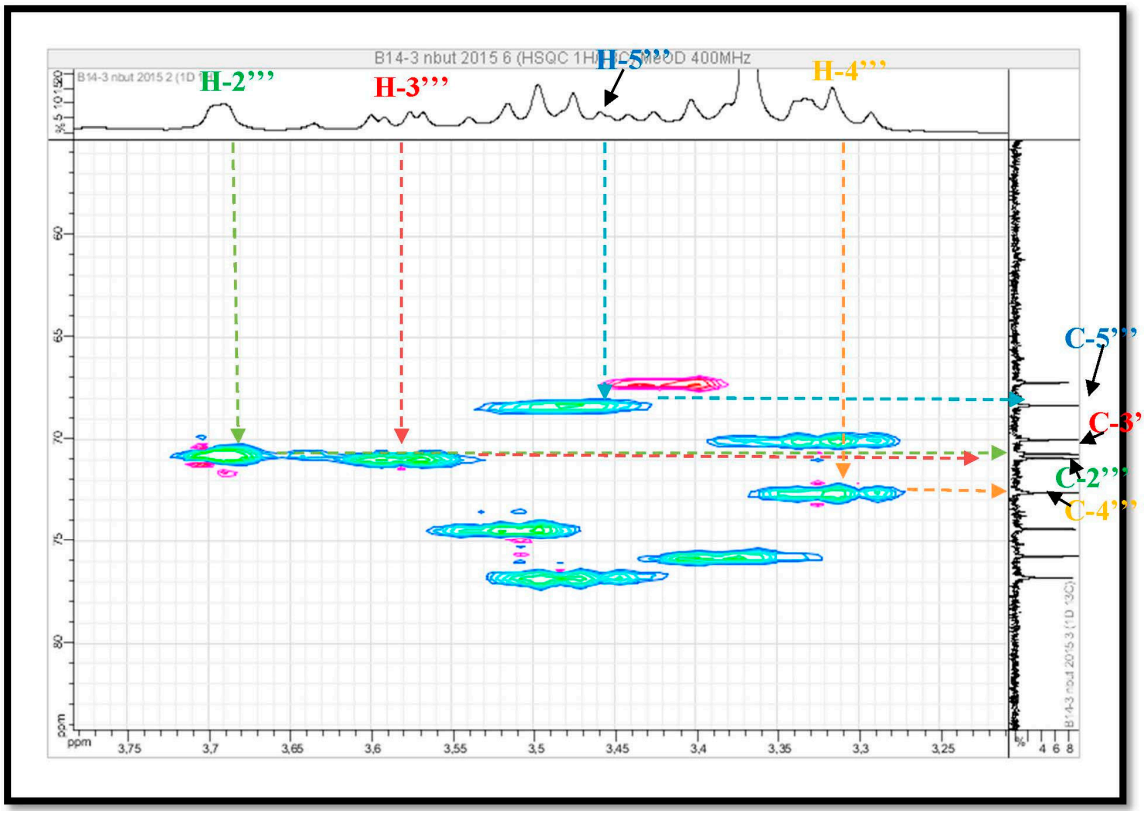


Figure S89. HMBC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of rutin.

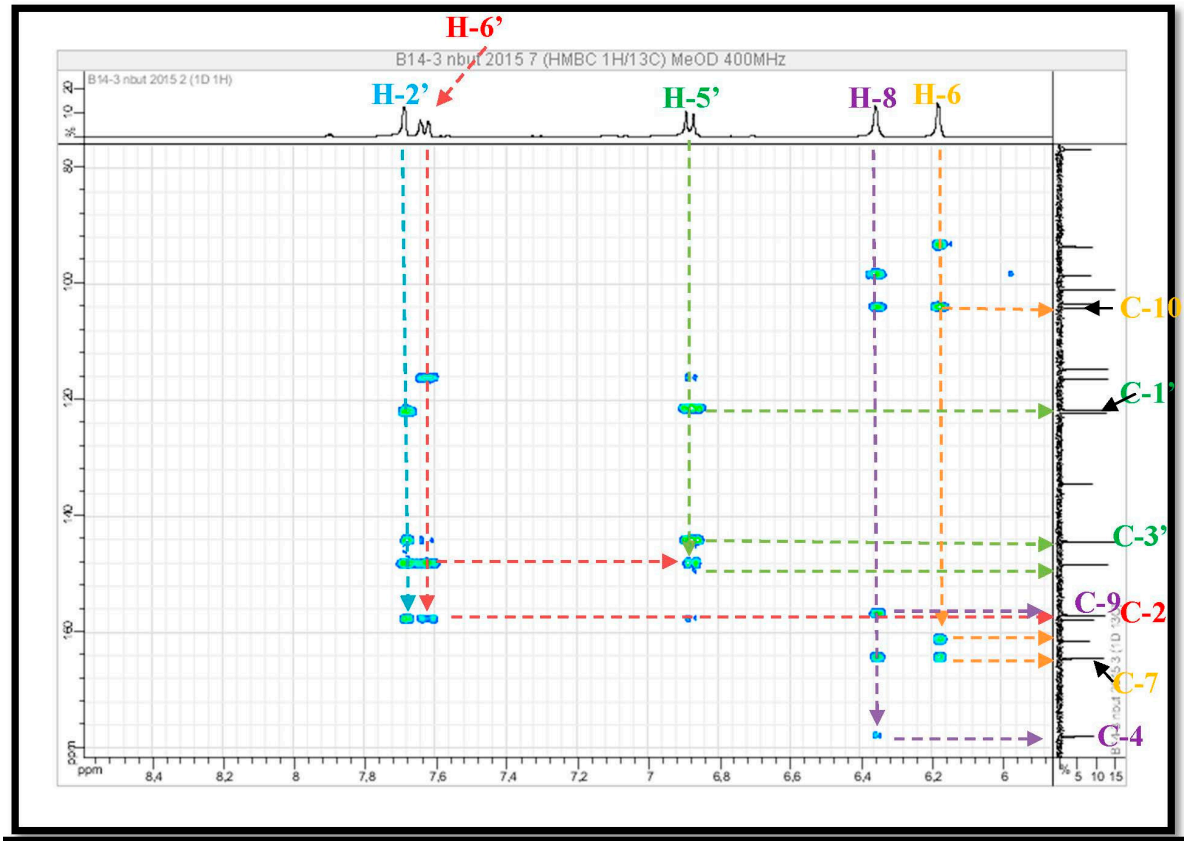


Figure S90. HMBC NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of rutin.

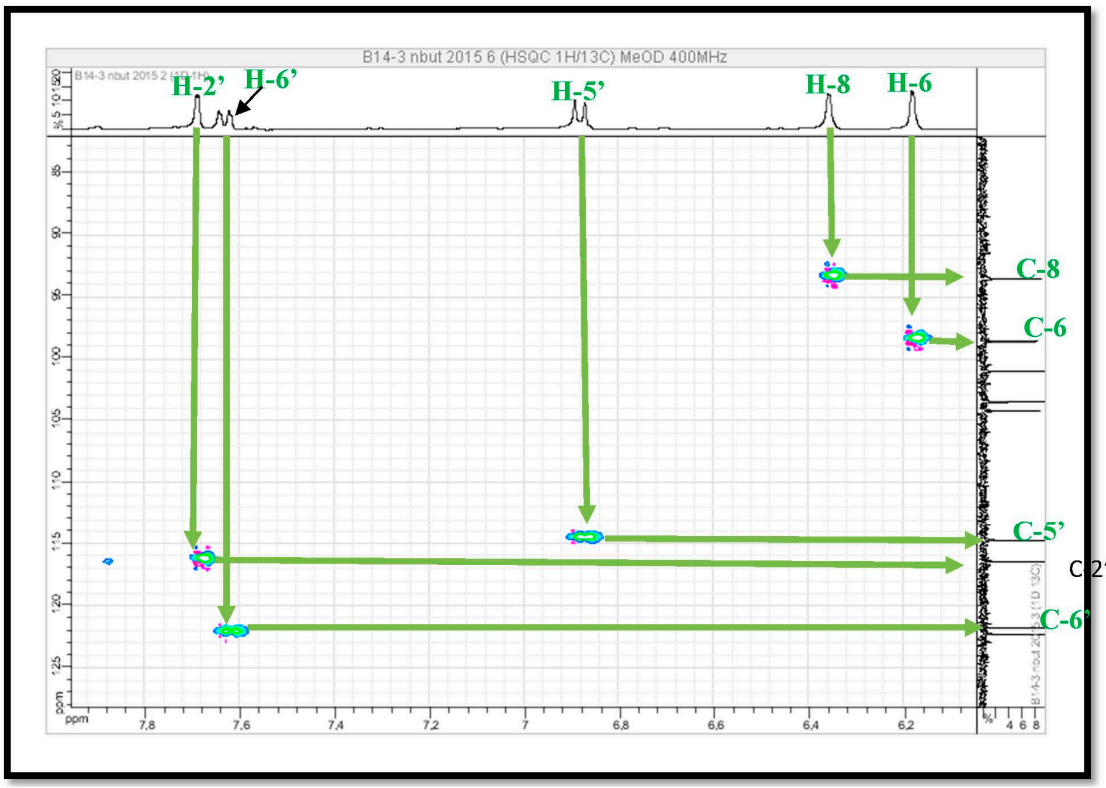


Figure S91. HMBC NMR spectrum (spreading out 4) (400 MHz, CD_3OD, δppm) of rutin.

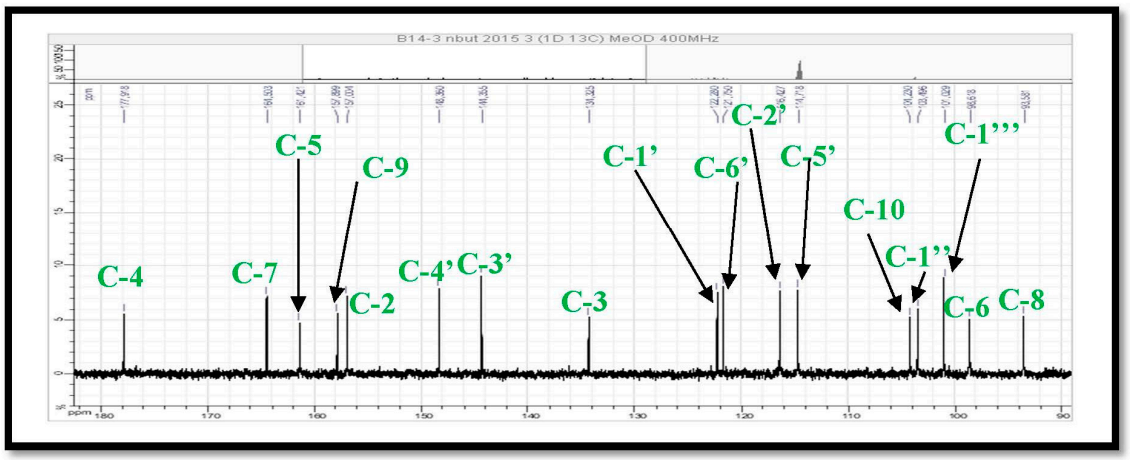
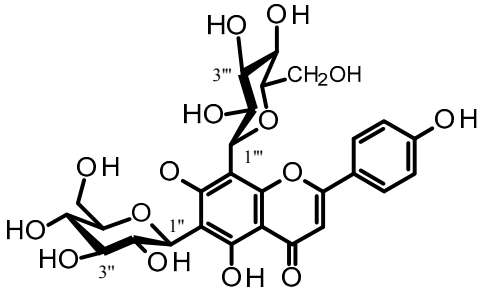


Figure S92. ^{13}CNMR spectrum (100 MHz, CD_3OD, δppm) of rutin.

Molecule 13: Vicenin-2



13

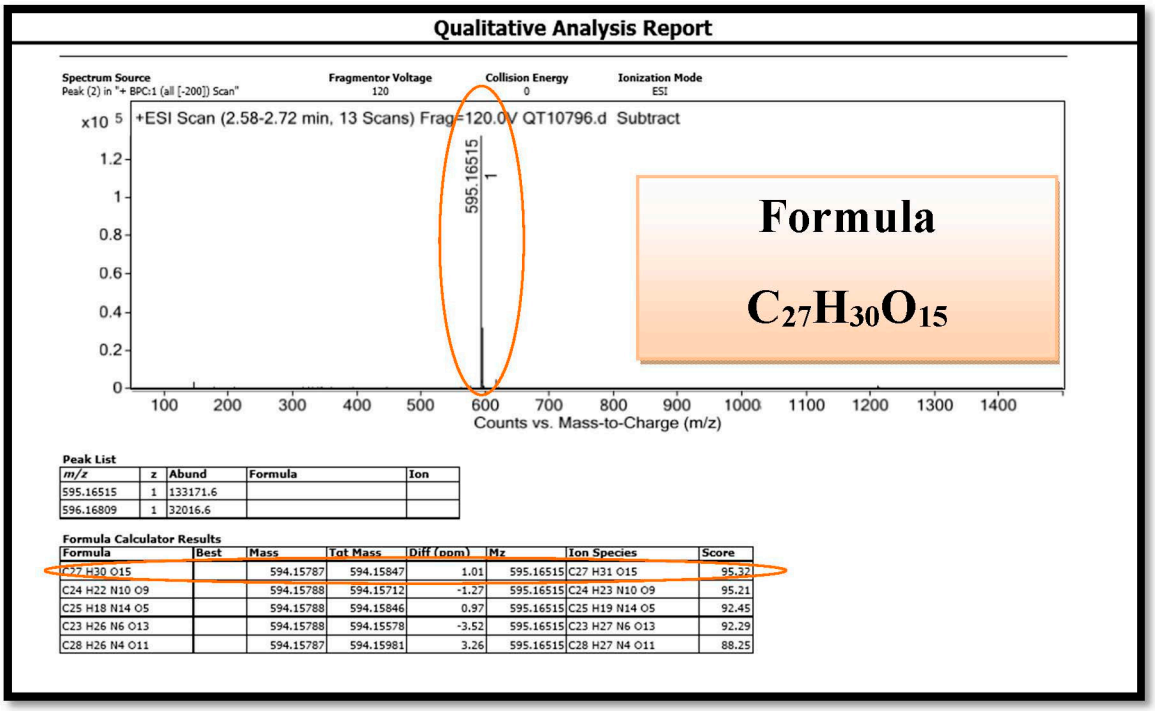


Figure S93. ESI-HRMS(+) of vicenin-2.

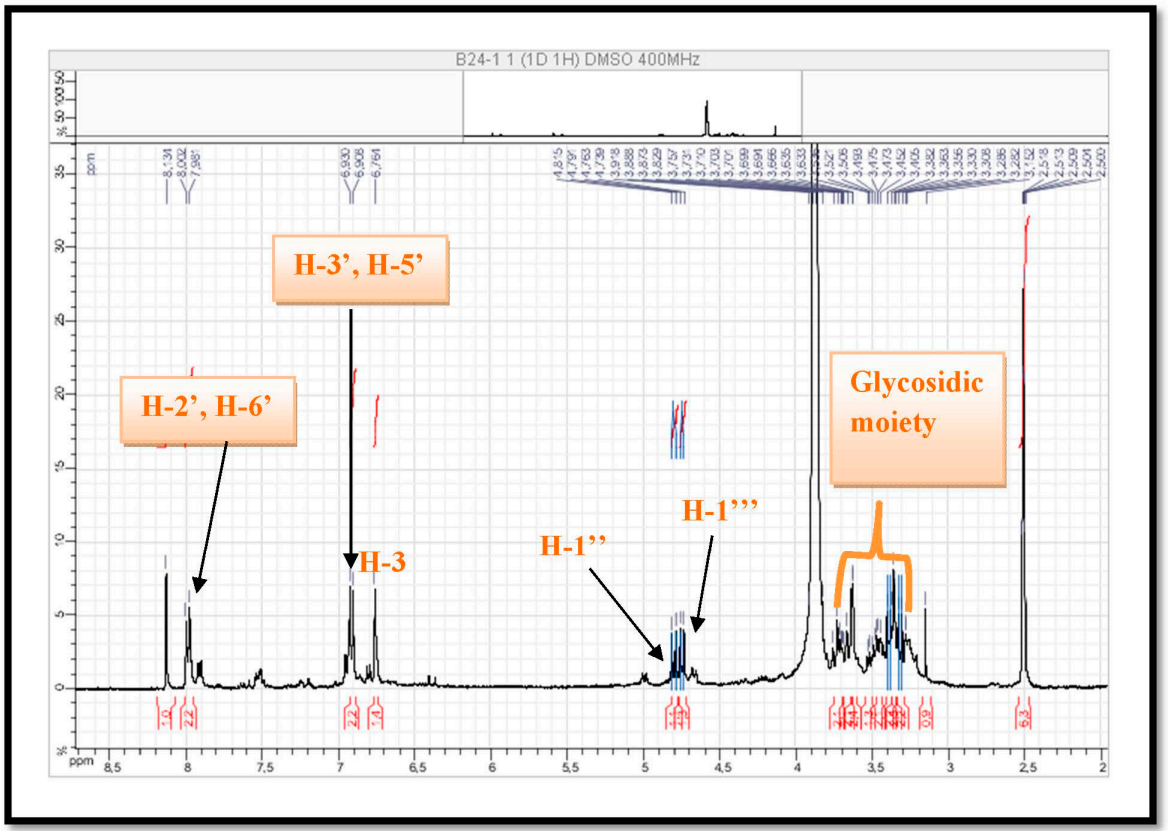


Figure S94. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δppm) of vicenin-2.

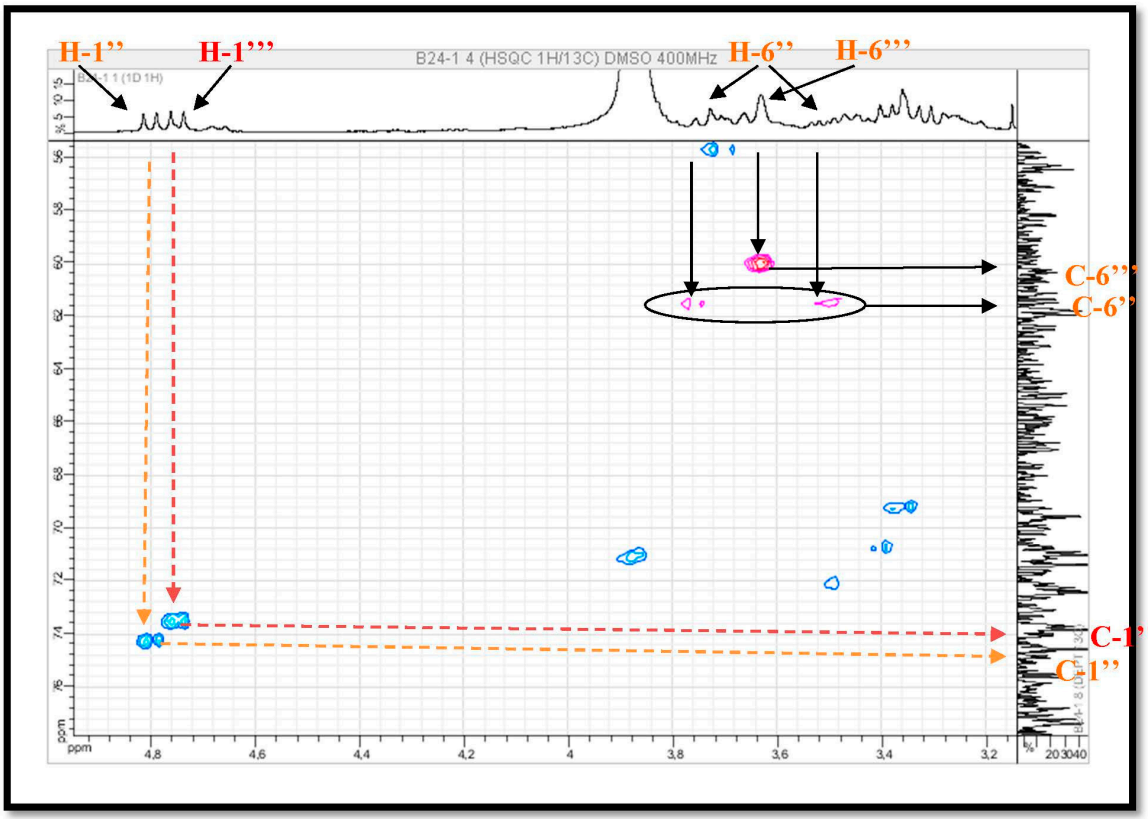


Figure S95. HSQC NMR spectrum (spreading out 1) (400 MHz, DMSO-d_6, δppm) of vicenin-2.

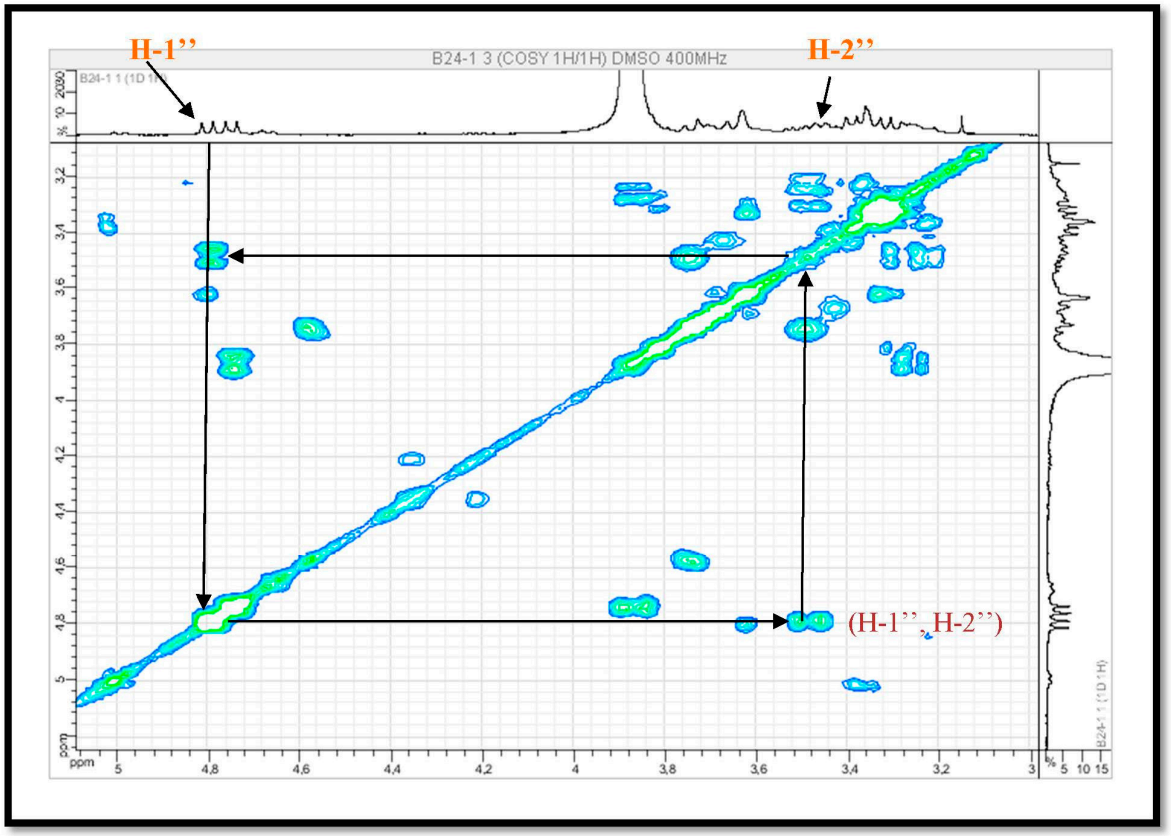


Figure S96. COSY NMR spectrum (spreading out 1) (400 MHz, DMSO-d_6, δppm) of vicenin-2.

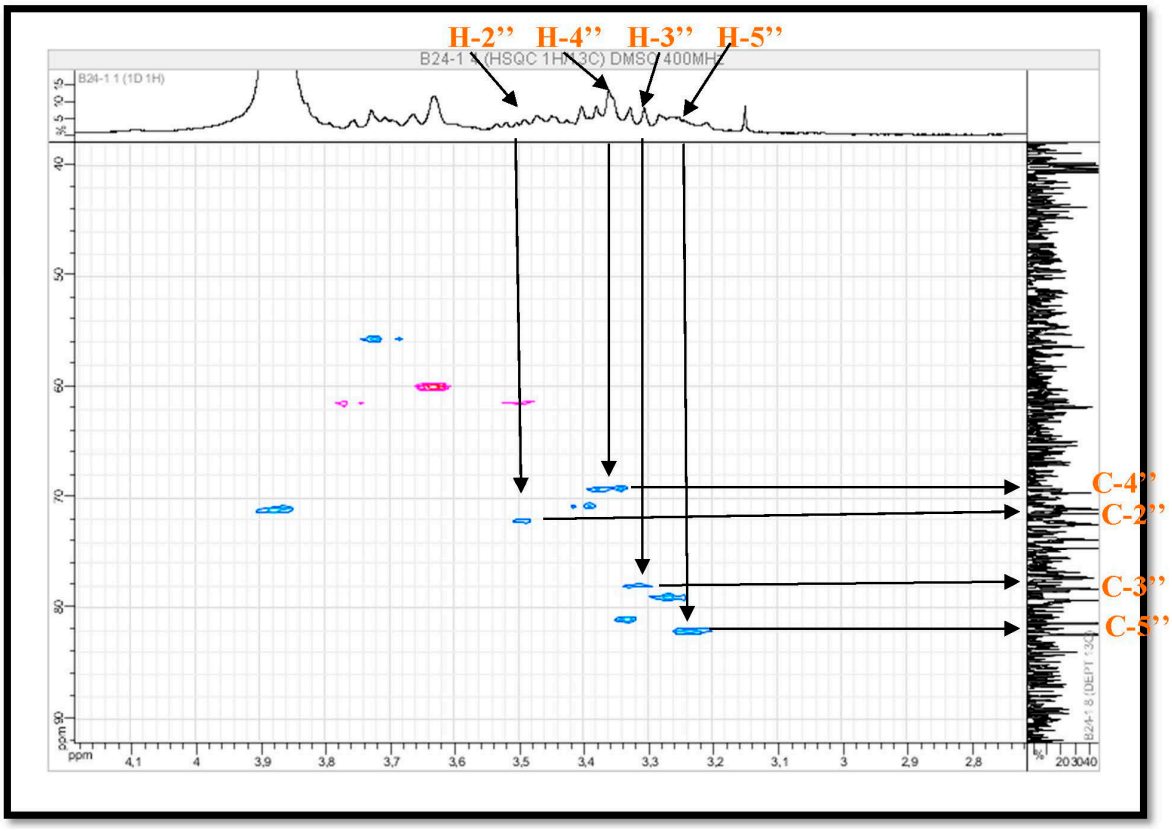


Figure S97. HSQC NMR spectrum (spreading out 2) (400 MHz, DMSO-d_6, δppm) of vicenin-2.

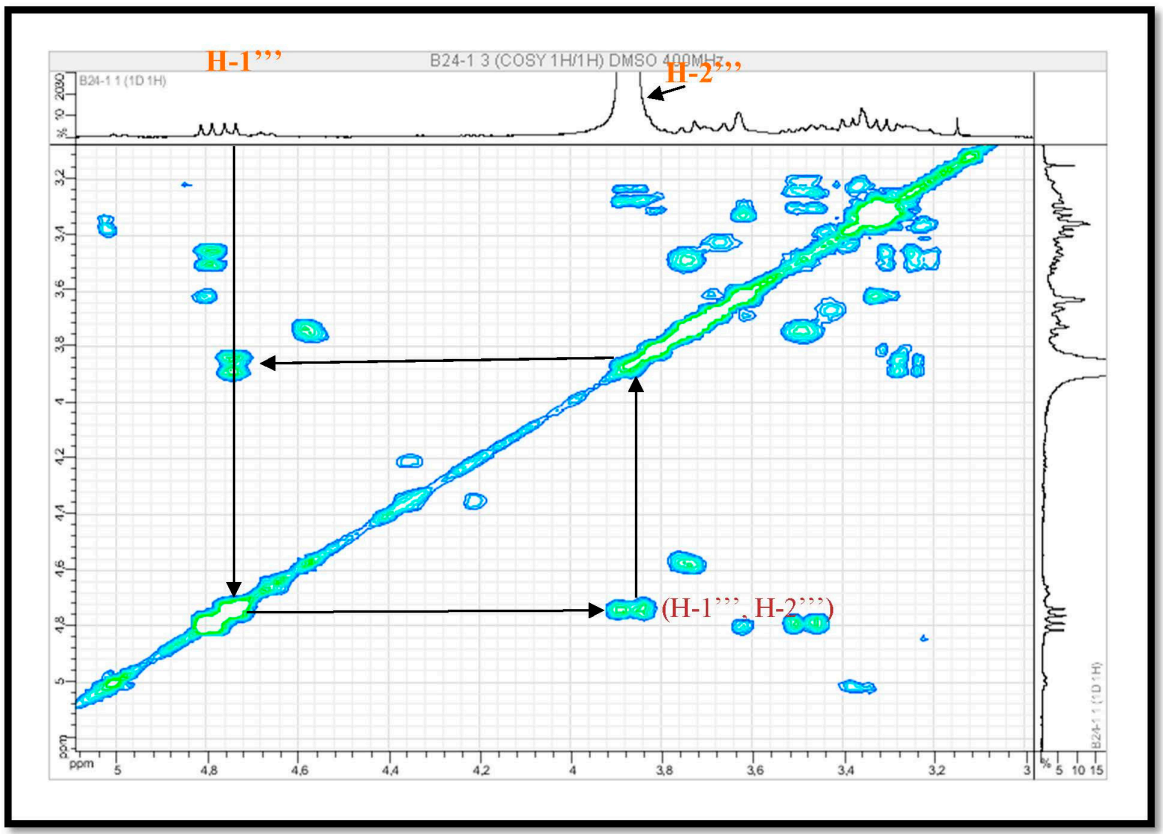


Figure S98. COSY NMR spectrum (spreading out 2) (400 MHz, DMSO-d_6, δppm) of vicenin-2.

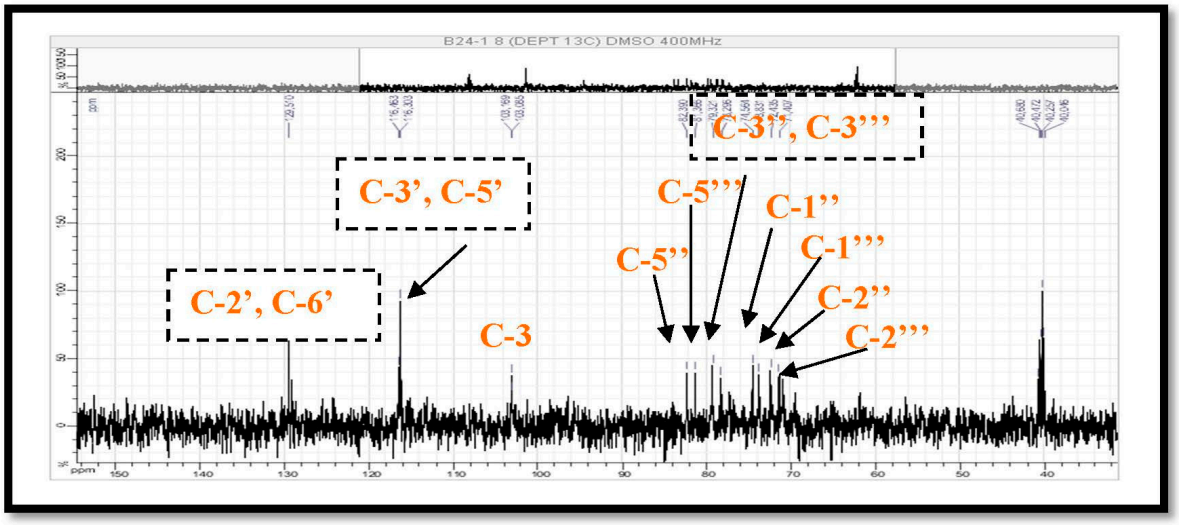


Figure S99. ^{13}C NMR DEPT spectrum (100 MHz, DMSO-d_6, δppm) of vicenin-2.

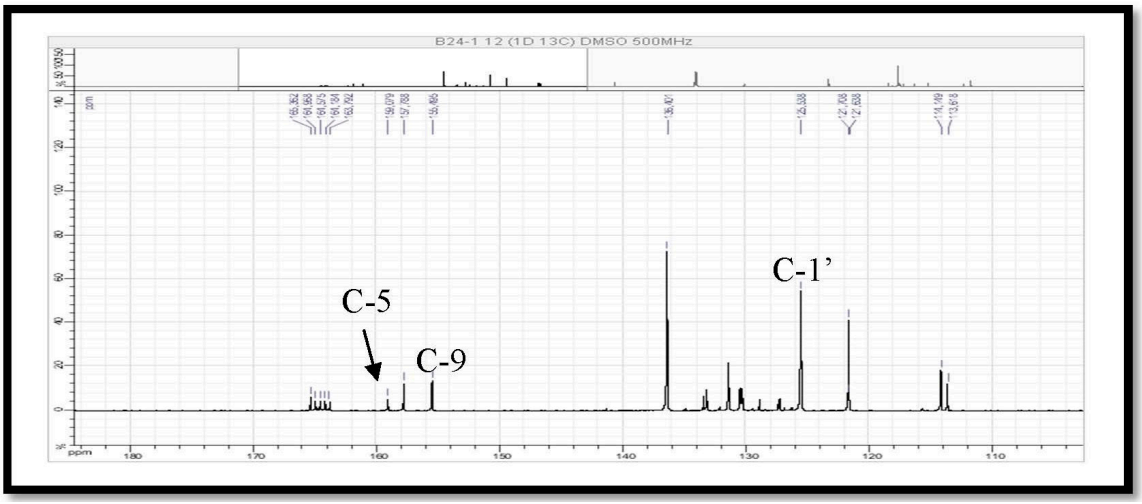


Figure S100. ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δppm) of vicenin-2.

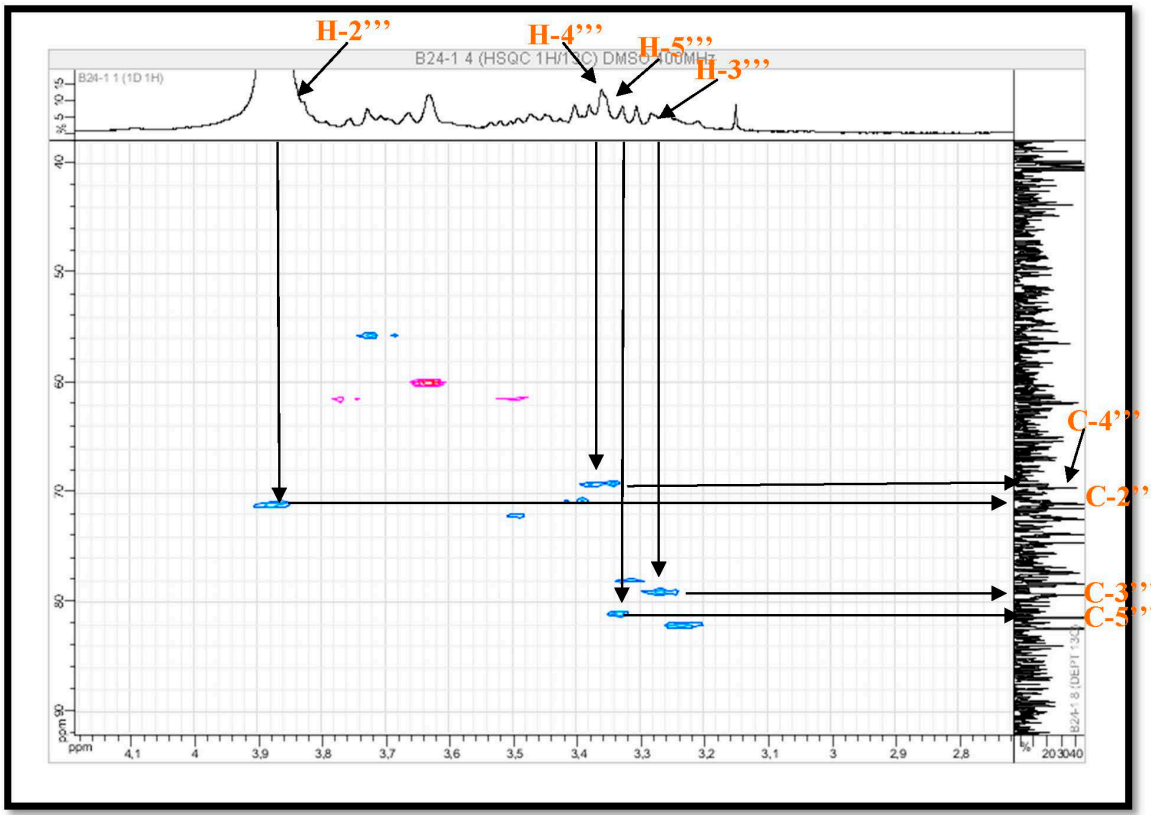


Figure S101. HSQC NMR spectrum (spreading out 3) (400 MHz, DMSO-d_6, δppm) of vicenin-2.

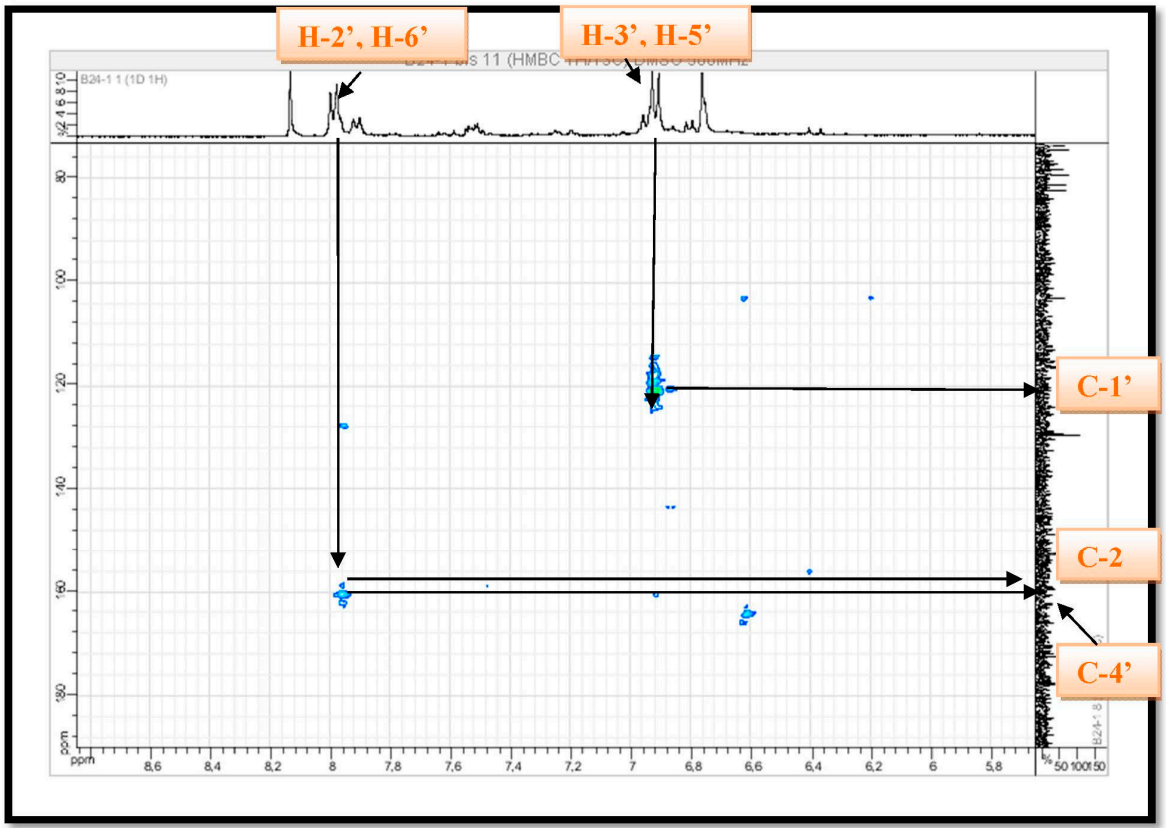


Figure S102. HMBC NMR spectrum (spreading out 1) (400 MHz, DMSO-d_6, δppm) of vicenin-2.

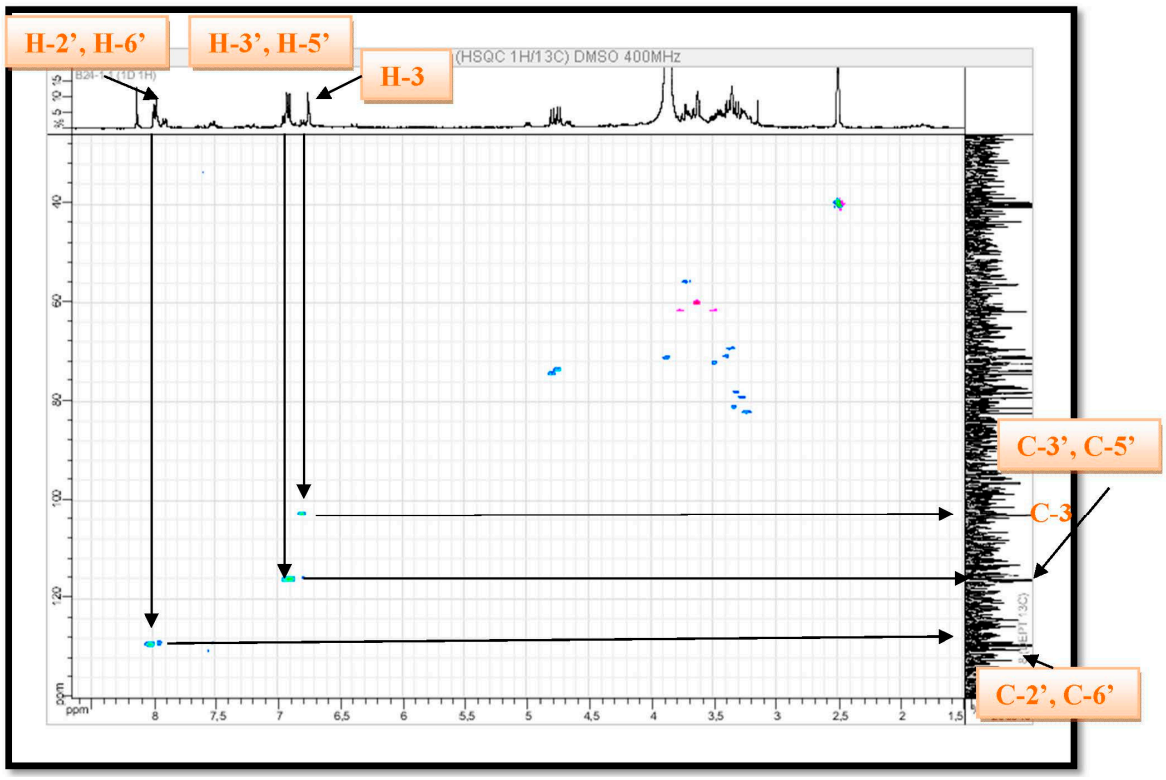
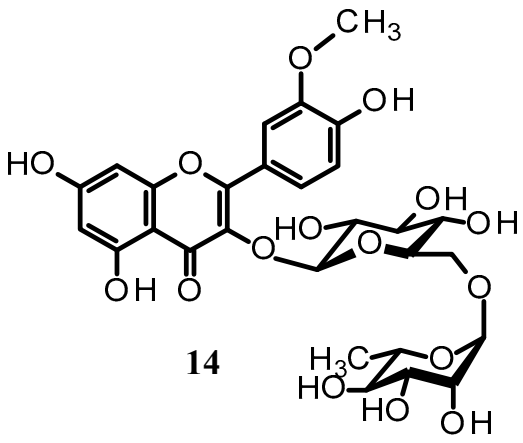
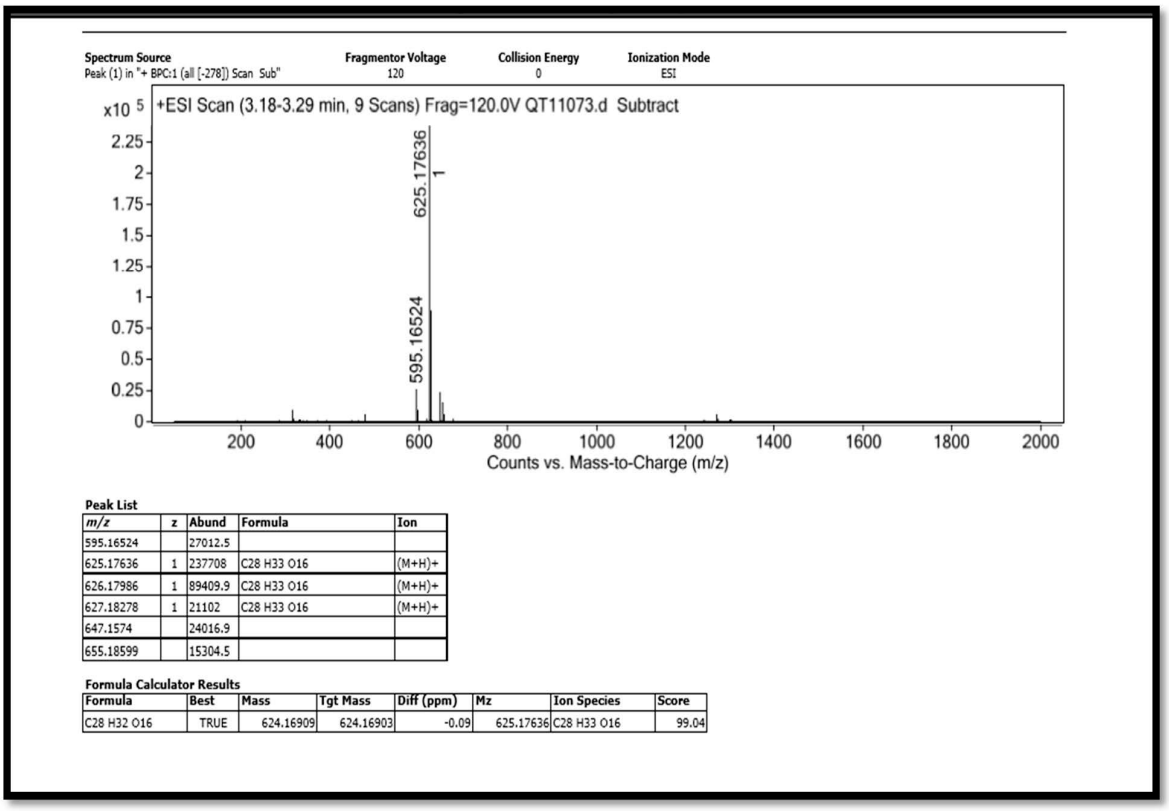


Figure S103. HSQC NMR spectrum (spreading out 4) (400 MHz, DMSO-d_6, δppm) of vicenin-2.

Molecule 14: Narcissin



14



Peak List

m/z	z	Abund	Formula	Ion
595.16524		27012.5		
625.17636	1	237708	C28 H33 O16	(M+H)+
626.17986	1	89409.9	C28 H33 O16	(M+H)+
627.18278	1	21102	C28 H33 O16	(M+H)+
647.1574		24016.9		
655.18599		15304.5		

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Mz	Ion Species	Score
C28 H32 O16	TRUE	624.16909	624.16903	-0.09	625.17636	C28 H33 O16	99.04

Figure S104. ESI-HRMS(+) of narcissin.

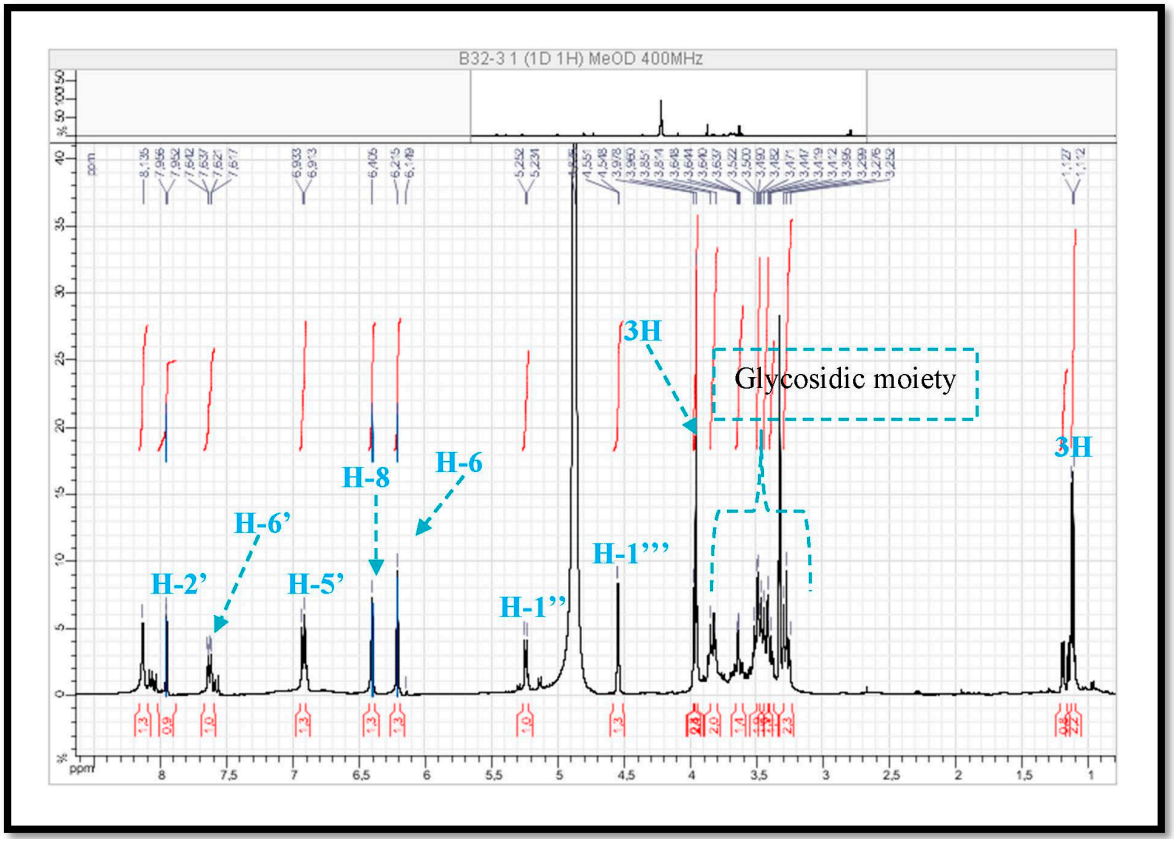


Figure S105. ^{1}H NMR spectrum (400 MHz, CD_3OD, δppm) of narcissin.

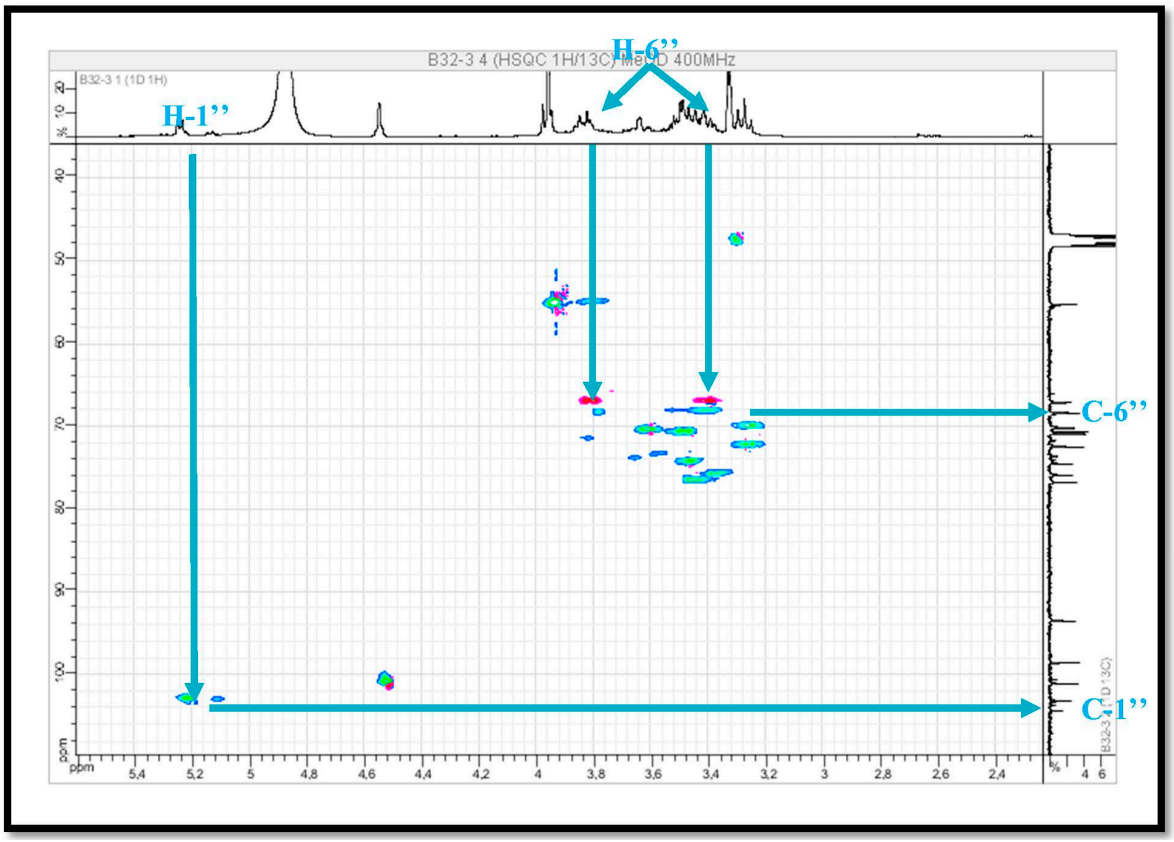


Figure S106. HSQC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of narcissin.

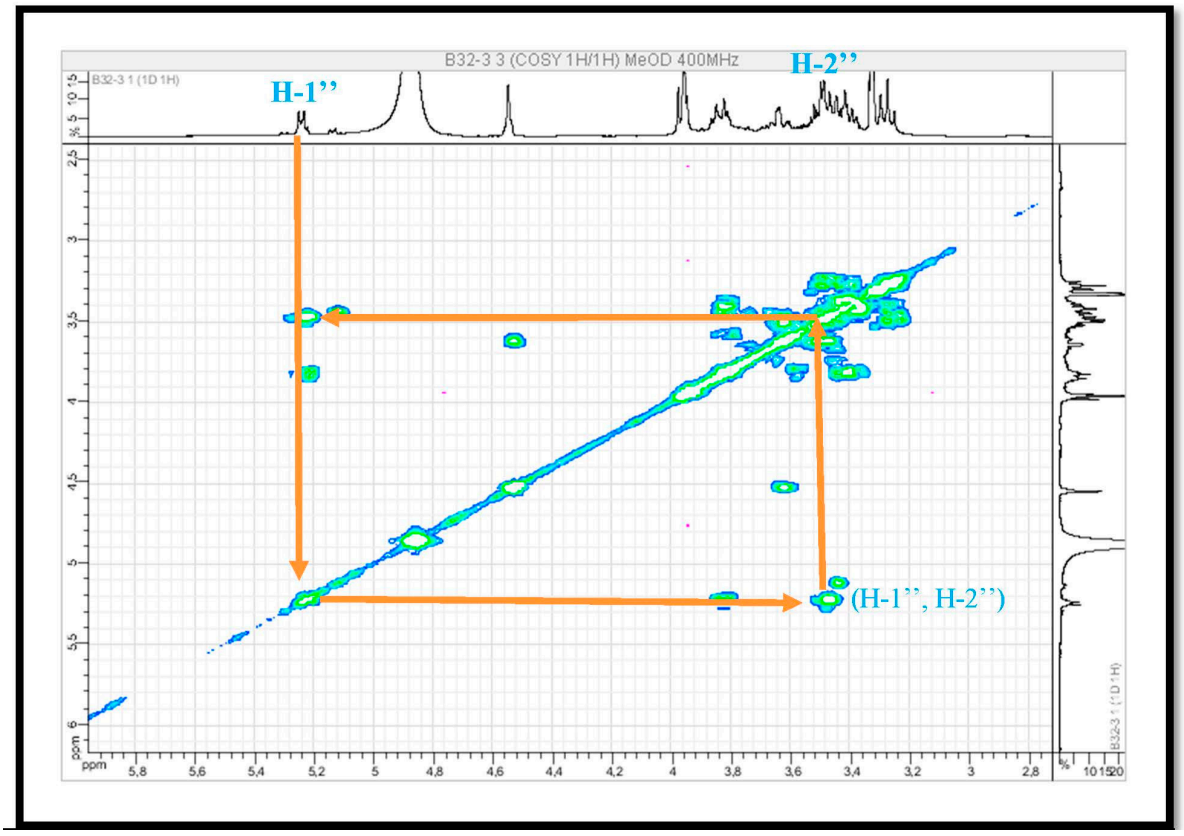


Figure S107. COSY NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of narcissin.

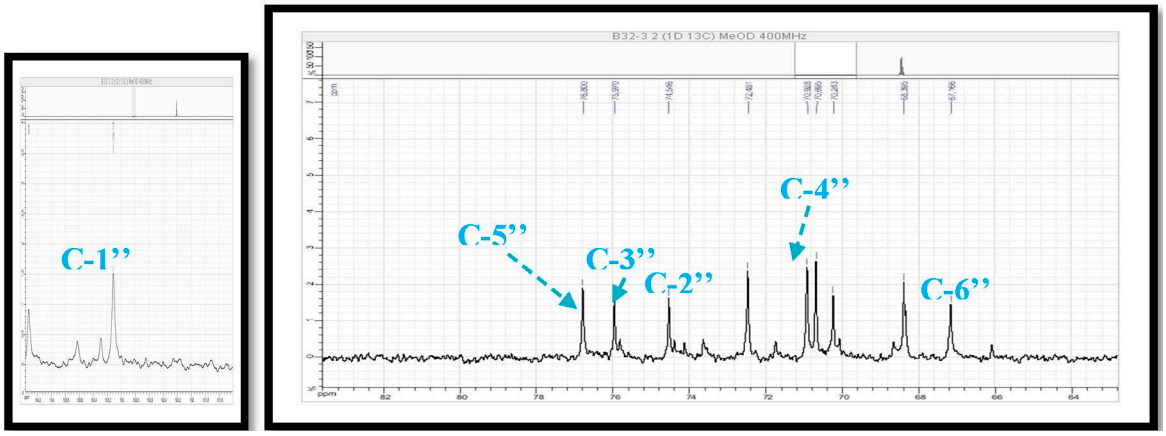


Figure S108. ^{13}C NMR spectrum (spreading out 1) (100 MHz, CD_3OD, δppm) of narcissin.

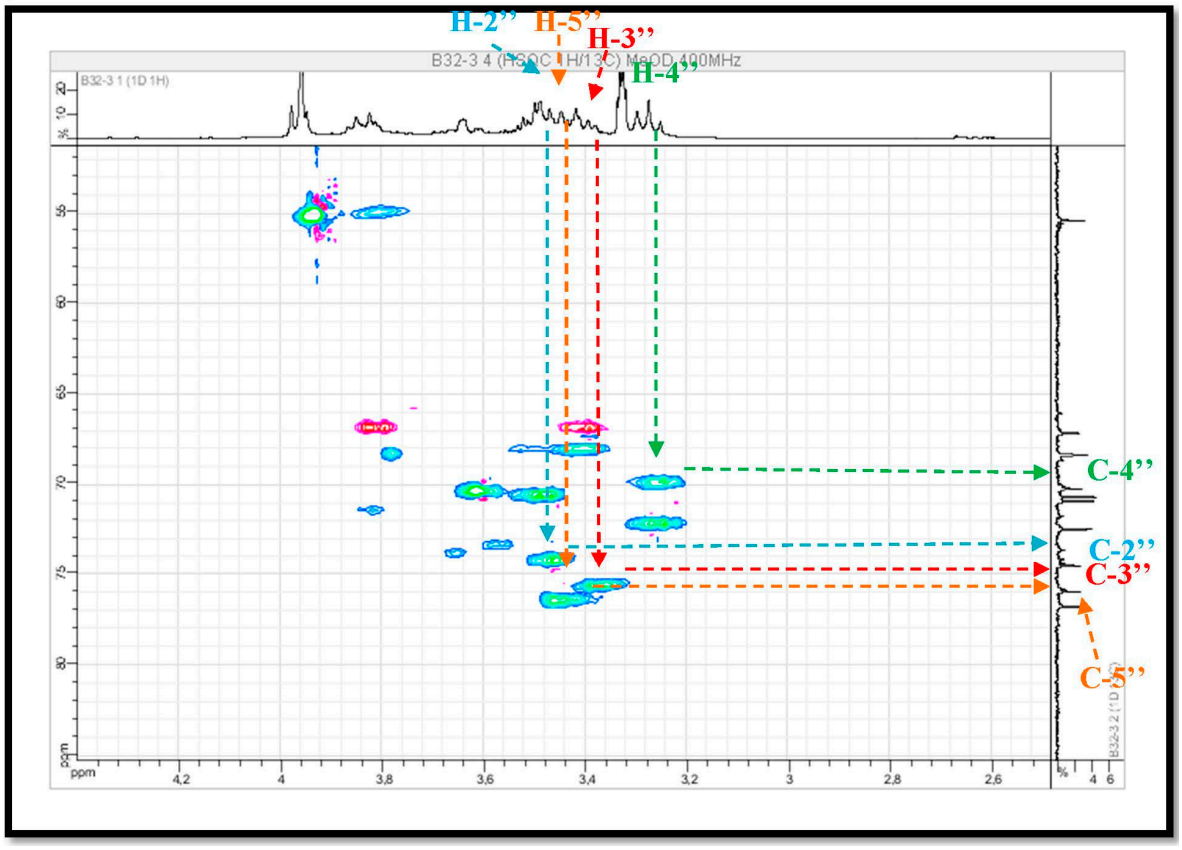


Figure S109. HSQC NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of narcissin.

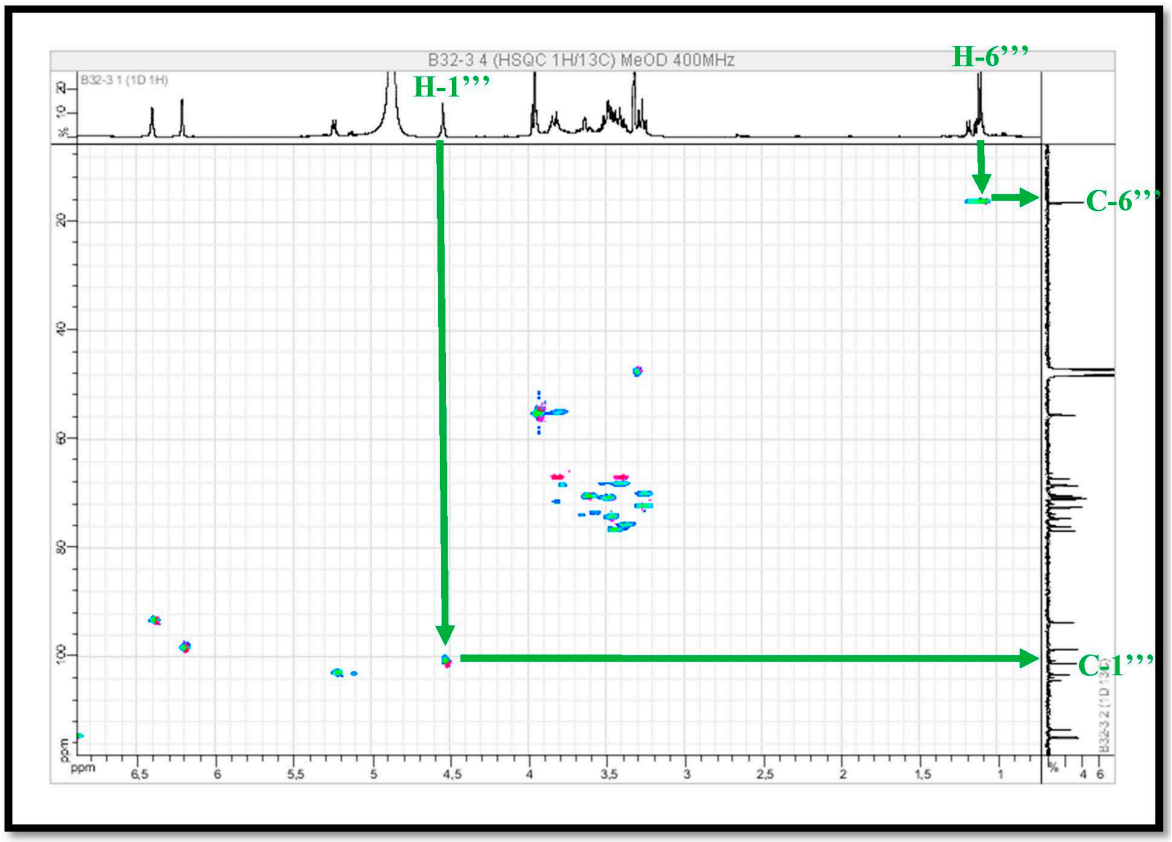


Figure S110. HSQC NMR spectrum (spreading out 3) (400 MHz, CD_3OD, δppm) of narcissin.

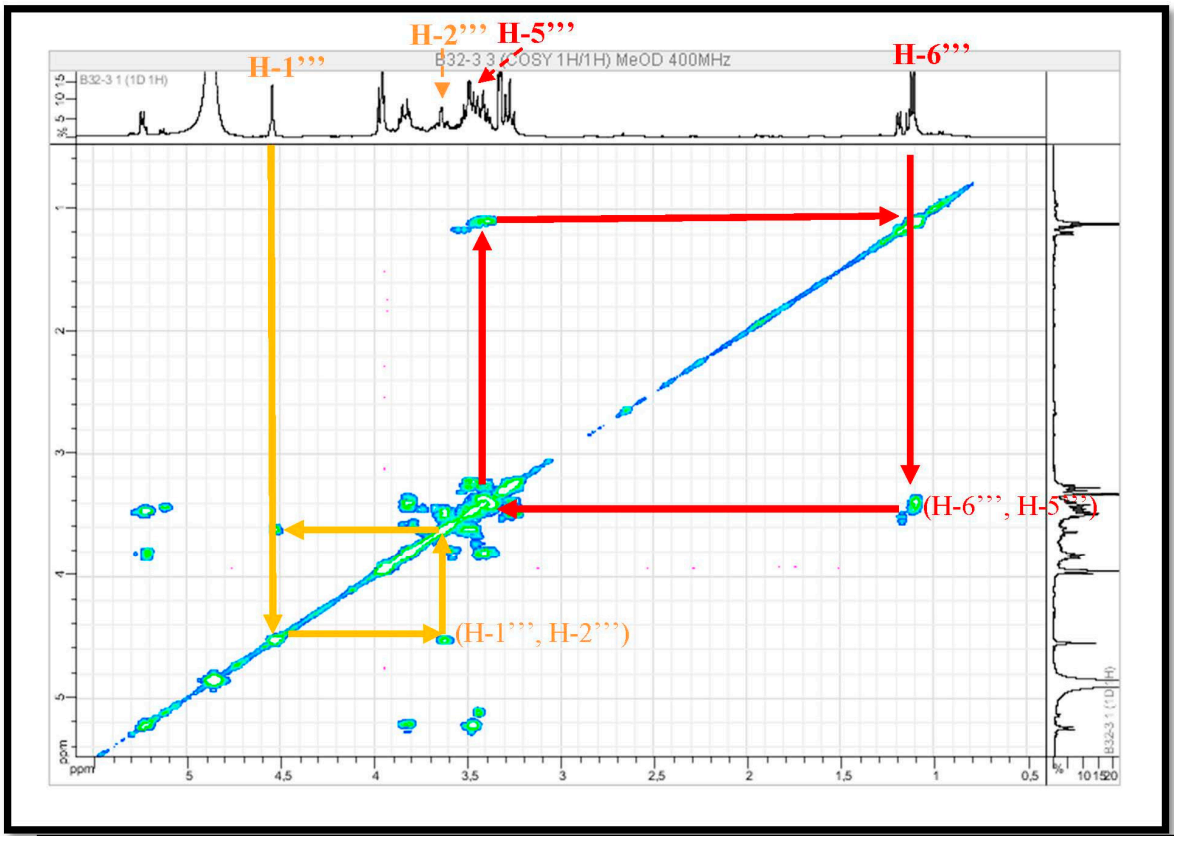


Figure S111. COSY NMR spectrum (spreading out 2) (400 MHz, CD_3OD, δppm) of narcissin.

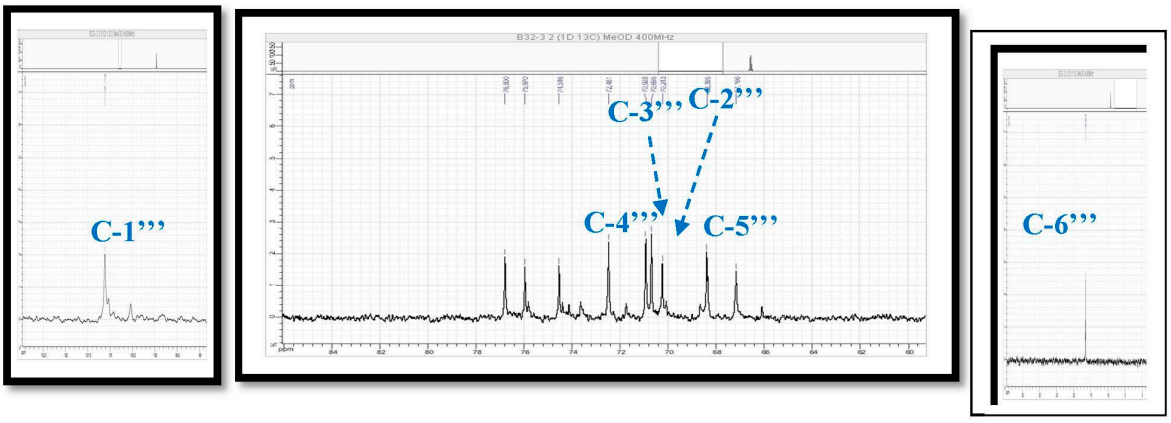


Figure S112. ^{13}C NMR spectrum (spreading out 2) (100 MHz, CD_3OD, δppm) of narcissin.

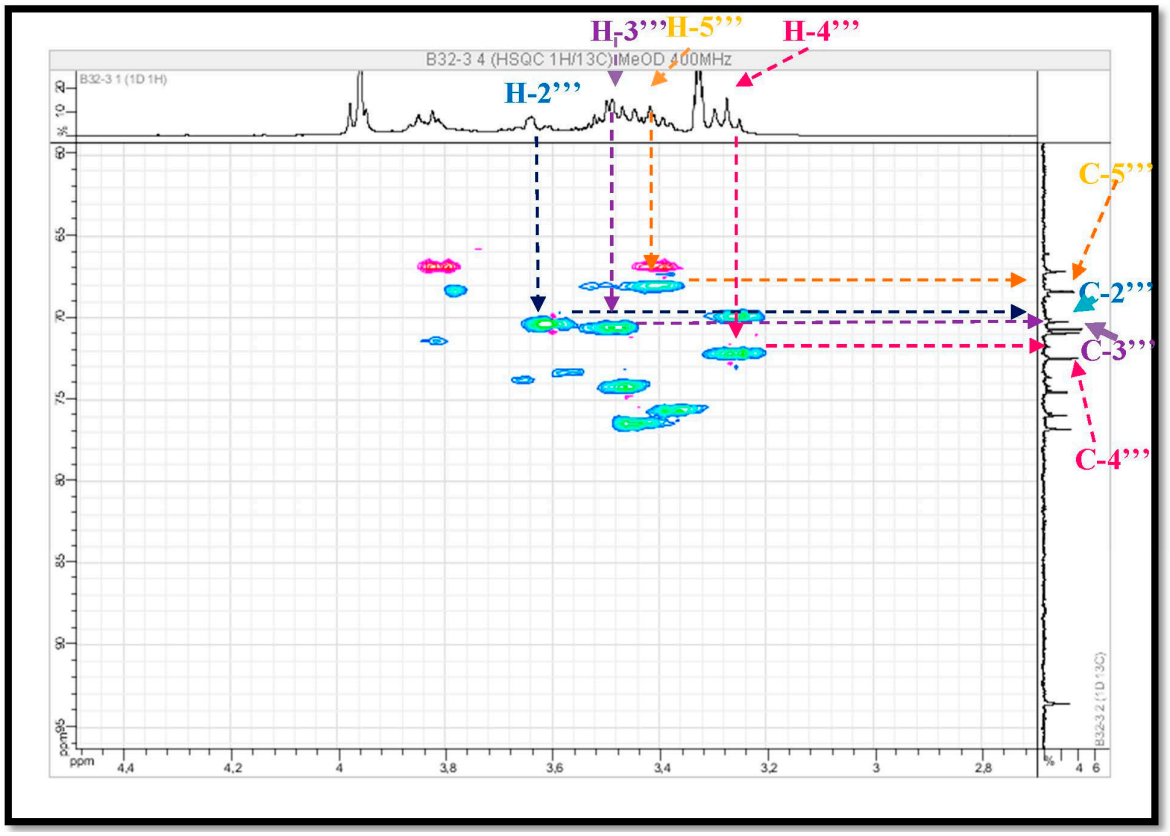


Figure S113. HSQC NMR spectrum (spreading out 4, 400 MHz, CD_3OD, δppm) of narcissin.

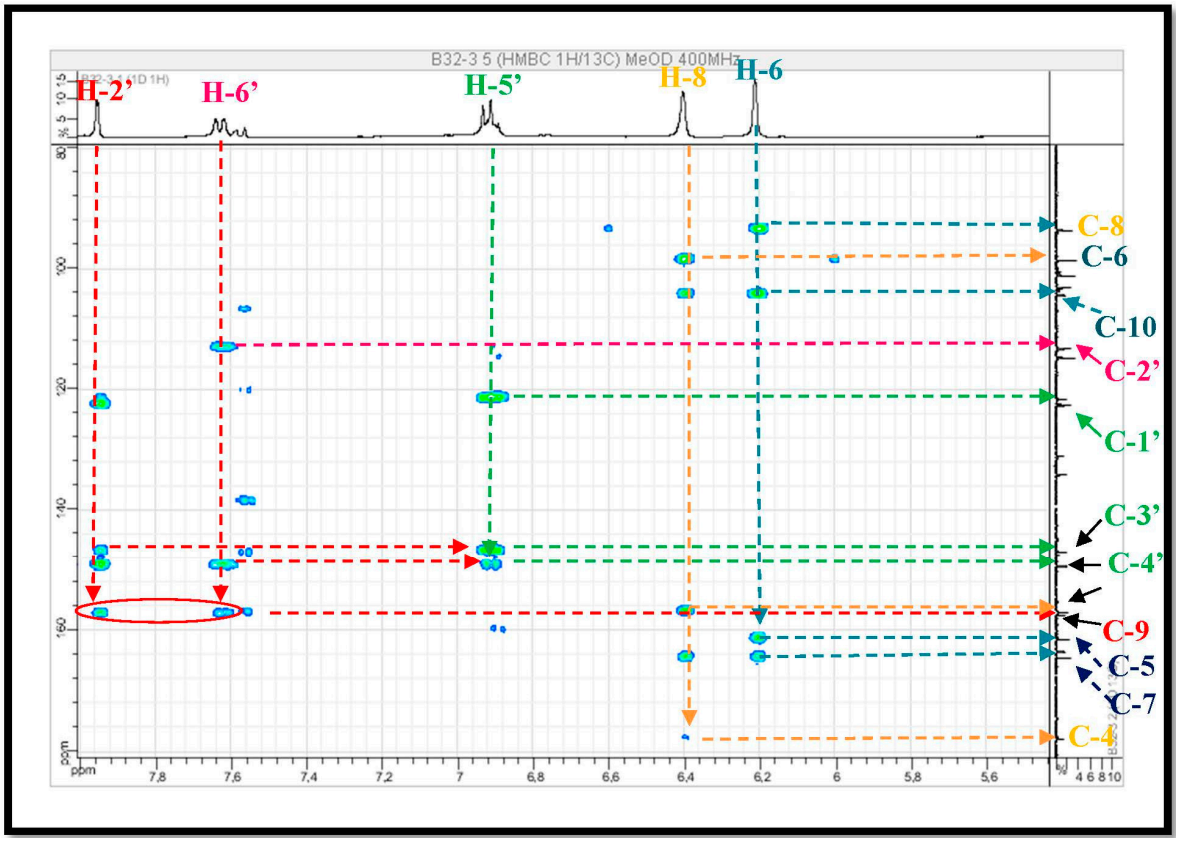


Figure S114. HMBC NMR spectrum (spreading out 1) (400 MHz, CD_3OD, δppm) of narcissin.

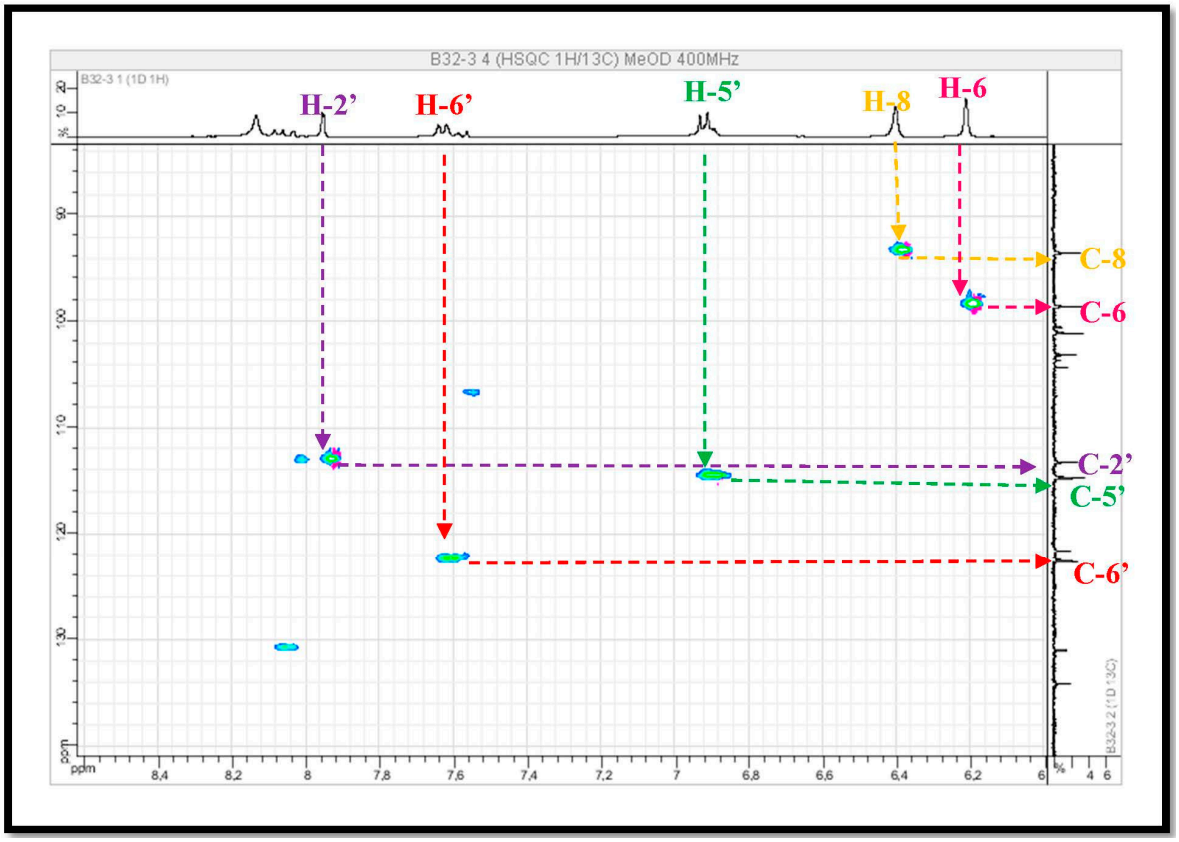


Figure S115. HSQC NMR spectrum (spreading out 5) (400 MHz, CD_3OD, δppm) of narcissin.

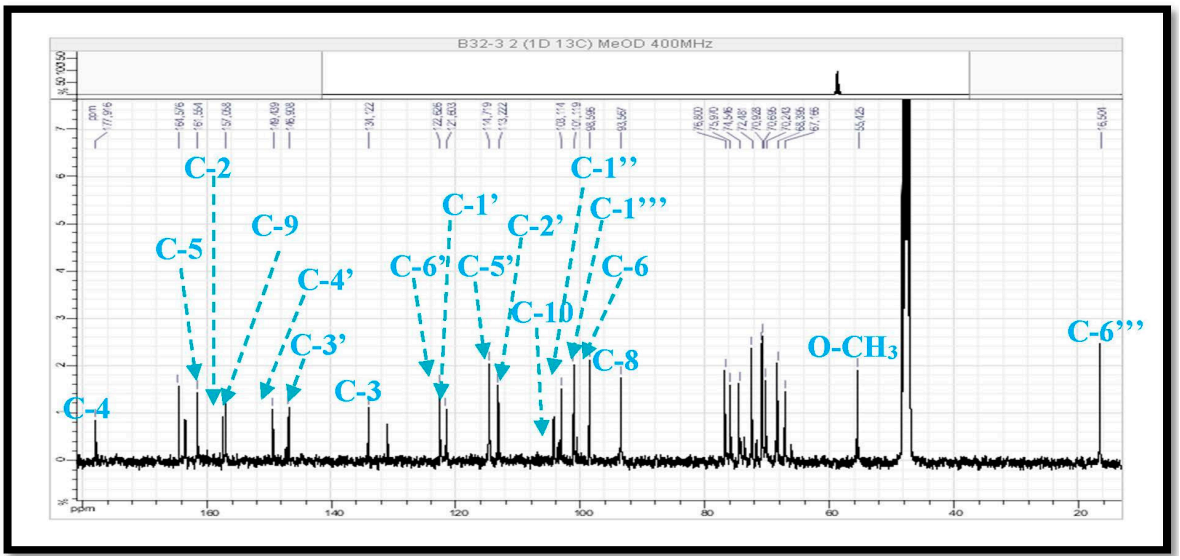


Figure S116. ^{13}C NMR spectrum (spreading out 3) (100 MHz, CD_3OD, δppm) of narcissin.

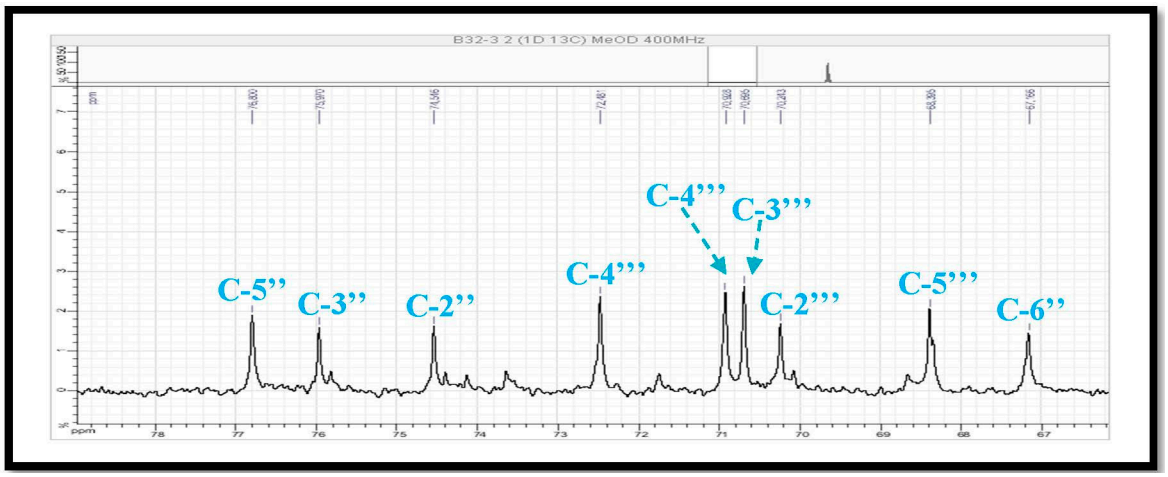


Figure S117. ^{13}C NMR spectrum (spreading out 4) (100 MHz, CD_3OD, δppm) of narcissin.