

New Flavone from the Leaves of *Neea theifera* (Nyctaginaceae)

Daniel Rinaldo, Clenilson M. Rodrigues, Juliana Rodrigues, Miriam Sannomiya,
Lourdes C. dos Santos and Wagner Vilegas*

Instituto de Química, Universidade Estadual Paulista, CP 355, 14801-900 Araraquara-SP, Brazil

Neea theifera Oerst. (Nyctaginaceae) é amplamente utilizada na medicina popular brasileira para tratamento de úlceras gástricas e inflamação. A investigação fitoquímica das folhas de *Neea theifera* permitiu o isolamento e identificação da nova flavona luteolina-7-*O*-[2"-*O*-(5"-*O*-feruloil)- β -D-apiofuranosil]- β -D-glucopiranosídeo (**1**) além dos oito compostos conhecidos vitexina, isovitexina, isoorientina, orientina, vicenina-2, crisoeriol, apigenina e luteolina. A identificação química foi realizada por métodos espectroscópicos incluindo RMN-2D, bem como análises no UV e IES-EM.

Neea theifera Oerst. (Nyctaginaceae) is widely used in Brazilian folk medicine for the treatment of gastric ulcers and inflammation. Phytochemical investigation of the leaves of *Neea theifera* afforded the isolation of the new flavone luteolin-7-*O*-[2"-*O*-(5"-*O*-feruloyl)- β -D-apiofuranosyl]- β -D-glucopyranoside (**1**) besides the eight-known compounds vitexin, isovitexin, isoorientin, orientin, vicenin-2, chrysoeriol, apigenin and luteolin. Their chemical identification was established by NMR spectroscopic methods including 2D-NMR, as well as UV and ESI-MS analyses.

Keywords: *Neea theifera*, Nyctaginaceae, flavone, luteolin-7-*O*-[2"-*O*-(5"-*O*-feruloyl)- β -D-apiofuranosyl]- β -D-glucopyranoside

Introduction

The Nyctaginaceae family comprises around 300 species in 30 genera.¹ Phytochemical investigation with plants from this family is still scarce. Previous phytochemical studies of *Boerhavia coccinea* and *B. erecta* led to the isolation of tannins and saponins,² while *B. diffusa* yielded dihydroisofuranoxanthone,³ rotenoids⁴ and lignans.⁵ Betacianins and flavonoids were isolated from *Bougainvillea glabra*,⁶ already flavonoids and phenolic compounds were isolated from *B. spectabilis*.^{7,8} Saponins were isolated from *Colignonia scandens* Benth.⁹ and from *Pisonia umbellifera*.¹⁰

In our search for bioactive natural products from São Paulo State, Brazil, we have examined the leaves of *Neea theifera* Oerst. This species is popularly known as 'Caparosa-do-campo' that grow wild in cerrado lands of Brazil.¹ They are used in folk medicine for the treatment of gastric ulcers and inflammation.¹¹ To the best of our knowledge, we could not find any previous phytochemical investigation with plants belonging to this genus.

The present paper describes the isolation, purification and structure elucidation of the nine compounds from the leaves of *N. theifera*.

Experimental

Plant material

Neea theifera Oerst. was collected in March 2005, at Corumbataí, Itirapina city, São Paulo State, Brazil, and authenticated by Prof. Dr. Jorge Yoshio Tamashiro from the Instituto de Biologia, Unicamp, São Paulo. A voucher specimen (HUEC 1396) is on file of the Herbarium of the Universidade Estadual de Campinas, Brazil.

Extraction and isolation

The dried leaves of *N. theifera* (953.5 g) were powdered and extracted successively with CHCl₃ and MeOH. The methanolic extract (3.0 g) was subjected to RP-C18 CC (40.0 cm \times 2.5 i.d.) eluted with H₂O:MeOH (9:1), H₂O:MeOH (1:1) and MeOH giving 3 fractions: fr. 9:1 (1.8 g), fr. 1:1 (0.86 g) and fr. MeOH (0.34 g),

*e-mail: vilegasw@gmail.com

respectively. The fr. 1:1 was chromatographed on Sephadex LH-20 with H₂O:MeOH (1:1) as eluent. Fractions (4.0 mL) were collected and checked by TLC [Si gel plates, CHCl₃:MeOH:*n*-PrOH:H₂O (5:6:1:4), organic phase] giving 426 fractions. Fractions 53-58, 59-61, 66-68, 162-170, 190-206, 233-254 and 284-301 were identified as vitexin (12 mg), isovitexin (26 mg), orientin (16 mg), vicenin-2 (8 mg), chrysoeriol (5 mg), apigenin (4 mg) and luteolin (4 mg), respectively. Fraction 74-82 (60 mg) was further purified by semi-preparative HPLC and afforded to obtain pure isoorientin (6 mg) and pure compound (**1**) (8 mg).

General experimental procedures

Melting point was measured on a digital MQ APF-301 (Microquímica[®], Brazil) apparatus. UV spectrum was recorded on a HACH UV-Vis DR/4000 spectrophotometer in MeOH. IR spectrum was obtained using Shimadzu FT-IR 8300 spectrophotometer in KBr disk. NMR analyses and 2D experiments were run on Varian[®] INOVA 500 operating at 500 MHz for ¹H and 125 MHz for ¹³C (11.7 T), using TMS as internal standard. The ESI-MS spectra were obtained with a Fisons Platform spectrometer in negative mode (70 V). The samples were dissolved in MeOH and injected directly. HREIMS was performed by using an ultratOFQ-ESI-TOF Mass Spectrometer Bruker Daltonics[®] instrument. The compound (**1**) was dissolved in MeOH, mixed with the internal calibrant, and introduced directly into the ion source by direct infusion. TLC analyses were performed on silica gel 200 μm (Sorbent Technologies[®]) and visualized using UV light (254 and 365 nm).

Semi-preparative HPLC analysis

Semi-preparative HPLC analysis was obtained on a HPLC Varian[®] ProStar 210 system equipped with a Varian[®] 330 photodiode array detector with a Phenomenex[®] Luna(2) RP18 column (40 × 2.5 cm, 10 μg) and Reodyne injector of 100 μL.

A binary gradient elution system with solvent A (0.05% TFA in H₂O) and solvent B (0.05% TFA in CH₃CN) was applied with linear gradient formation initially with 68:32 (A:B) at 35 min, and then it was changed to 55:45 (A:B) at 5 min, and finally 55:45 (A:B) at 30 min. The flow-rate was 4.7 mL min⁻¹.

Luteolin-7-O-[2''-O-(5'''-O-feruloyl)-β-D-apiofuranosyl]-β-D-glucopyranoside

Yellow amorphous powder. mp 250-252 °C. UV

(MeOH) λ_{max}/nm: 206, 249 and 334. IR (KBr) ν_{max}/cm⁻¹: 3433, 1655 and 1605. ESI-MS *m/z*: 755 [M-H]⁻, 579 [M-E-feruloyl-H]⁻, 561 [M-E-feruloyl-H₂O-H]⁻, 447 [M-E-feruloyl-apiose-H]⁻, 285 [M-E-feruloyl-apiose-glucose-H]⁻. HRESIMS [M-H]⁻ *m/z* 755.1902 (calculated for C₃₆H₃₆O₁₈ -H, 755.1823). For ¹H and ¹³C NMR analyses see Table 1.

Results and Discussion

The methanolic extract of the dried powdered leaves of *N. theifera* was purified by column chromatography (CC) on a Sephadex LH-20 column followed by purification by HPLC-DAD, afforded the isolation of the new compound (**1**). In addition, eight-known compounds were identified by comparison of their spectroscopic properties with published data: vitexin, isovitexin, isoorientin, orientin, vicenin-2, chrysoeriol, apigenin and luteolin.¹²⁻¹⁴

Compound (**1**) was isolated as a yellow solid (mp 250-252 °C). The UV spectral data showed absorption bands at 249 nm and 334 nm. The IR spectrum presented bands at 3433 cm⁻¹ (OH), at 1655 cm⁻¹ (C=O) and 1605 cm⁻¹ (C=C).

The molecular formula of compound (**1**) was calculated as C₃₆H₃₆O₁₈ from its HRESIMS, which showed a [M-H]⁻ at *m/z* 755.1902 (calculated for C₃₆H₃₆O₁₈ -H, 755.1823). The negative ESI-MS (70 V) exhibited the pseudomolecular ion [M-H]⁻ at *m/z* 755. Key fragmentation ions occurred at *m/z* 579 [M-E-feruloyl-H]⁻, *m/z* 561 [M-E-feruloyl-H₂O-H]⁻, *m/z* 447 [M-E-feruloyl-apiose-H]⁻ and *m/z* 285 [M-E-feruloyl-apiose-glucose-H]⁻.

The ¹H NMR spectrum (Table 1) showed signals at δ 7.34 (1H, d, *J* 8.0 Hz), δ 7.35 (1H, brs) and at δ 6.88 (1H, d, *J* 8.0 Hz) assigned to H-6', H-2' and H-5' respectively, two doublets at δ 6.68 (1H, d, *J* 2.0 Hz) and δ 6.36 (1H, d, *J* 2.0 Hz), attributed to H-8 and H-6 of the A-ring, and one singlet at δ 6.54 (1H, s) attributed to H-3 typical of a luteolin derivative. Signals at δ 6.17 (1H, d, *J* 16 Hz, H-α), δ 7.30 (1H, d, *J* 16 Hz, H-β), δ 7.07 (1H, d, *J* 1.5 Hz, H-2'''), δ 6.69 (1H, d, *J* 8.0 Hz, H-5''') and δ 6.87 (1H, d, *J* 8.0 and 1.5 Hz, H-6''') suggested the presence of an *E*-feruloyl unit.¹⁵ A signal at δ 3.74 (3H, s) indicates the presence of a methoxyl group.¹³ NOESY experiment showed correlation between signal at δ 3.74 (OMe) and at δ 7.07, thus establishing the methoxyl group at position 3''' of the *E*-feruloyl unit.

A doublet at δ 5.20 (1H, d, *J* 7.5 Hz, H-1'') and a singlet at δ 5.38 (1H, s) in the ¹H NMR spectrum revealed the presence of two anomeric hydrogen from two sugar

units. The TOCSY experiment with irradiation at δ 5.20 displayed the spin system of the β -D-glucopyranoside unit, whereas irradiation at δ 5.38 resulted only in the singlet at δ 3.75 (1H, s) suggesting an apiofuranosyl unit. The coupling constant of the anomeric proton at δ 5.20 (1H, d, J 7.5 Hz) indicated that the present glucose unit has a β -configuration.¹³

The ^{13}C NMR experiment presented 35 signals, from which 15 were attributed to the aglycone, 9 to the *E*-feruloyl unit, 6 to the β -D-glucopyranosyl unit, and with 5 was possible determined a apiofuranosyl unit.¹⁴

The apiose unit was characterized through ^1H and ^{13}C NMR experiments compared to the literature data. In the ^1H NMR spectrum, apiose unit with OH linked to C-1''' and OH linked to C-2''' in *trans* configuration presents constant coupling $J_{1,2}$ 0-1 Hz, whereas *cis* configuration is characterized by $J_{1,2}$ 3-4 Hz.^{16,17} The chemical shift of C-1 in ^{13}C NMR experiments in pyridine- d_5 to the α -D-apiofuranoside is δ 105 and δ 112 to β -D-apiofuranoside,¹⁸ whereas in DMSO- d_6 these isomers produce signals at δ 108 and δ 109, respectively.^{19,20,21} Thus, the apiose unit in (**1**) was identified as being a β -D-apiofuranoside.

The structure and bonds of these units on compound (**1**) was established from gHMQC and gHMBC experiments. gHMQC experiment showed direct correlations between carbons and the respective hydrogens (Table 1). gHMBC experiments showed long-range correlations between the hydrogen signal at δ 5.20 (H-1'' glucose) and the carbon signal at δ 162.4 (C-7 aglycone), and between the hydrogen signal at δ 3.52 (H-2'' glucose) and the carbon signal at δ 108.2 (C-1''' apiose). Besides, the chemical shift of the C-2'' of the glucose (δ 75.8) unit was clearly deshielded (+3) compared to the chemical shift of the analogous carbon resonance of a non-substituted glucose unit (δ 72.9), supporting the glucose (1 \rightarrow 2) apiose linkage.¹⁴ The gHMBC experiment also showed

Table 1. NMR spectral data of compound **1** (11.7 T, DMSO- d_6)^a

Position	^1H NMR (δ_{H})	^{13}C NMR (δ_{C})	gHMBC (δ_{H})
2		164.4	H-3
3	6.54 s	103.0	
4		181.7	
5	12.91	161.2	H-6
6	6.36 d (2.0)	99.1	H-8
7		162.4	H-1''; H-6; H-8
8	6.68 d (2.0)	94.4	H-6
9		156.9	H-8
10		105.4	H-6; H-3; H-8
1'		121.6	
2'	7.35 brs	113.4	H-6'
3'		149.3	H-5'
4'		145.0	H-2'; H-6'
5'	6.88 d (8.0)	115.9	
6'	7.34 d (8.0)	119.1	
Glucose			
1''	5.20 d (7.5)	97.8	H-2''
2''	3.52 t (7.5; 7.5)	75.8	
3''	3.45 t (7.5; 7.5)	76.7	H-2''
4''	3.20 t (7.5; 7.5)	69.8	H-2''
5''	3.48 m	77.0	H-4''; H-3''
6''	a) 3.50 m b) 3.71 d (10.0)	60.5	
Apiose			
1'''	5.38 s	108.2	H-2'''; H-2''
2'''	3.75 s	76.6	
3'''		77.5	H-2'''; H-4'''
4'''	a) 3.78 d (9.5) b) 4.04 d (9.5)	73.8	H-5'''; H-2'''
5'''	4.06 brs	66.6	H-2'''; H-4'''
<i>E</i> -Feruloyl			
α	6.17 d (16.0)	113.7	H- β
β	7.30 d (16.0)	144.9	H-6'''; H-2'''
1''''		125.4	H- α ; H-5''''
2''''	7.07 d (1.5)	110.9	H-6'''; H- β
3''''		147.8	-OCH ₃ ; H-5''''
4''''		149.3	H-6'''; H-2'''
5''''	6.69 d (8.0)	115.4	H-6''''
6''''	6.87 dd (8.0; 1.5)	122.9	H-2'''; H- β
-OCH ₃	3.74 s	55.5	
C=O		166.3	H-5'''; H- β

^aChemical shifts (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses.

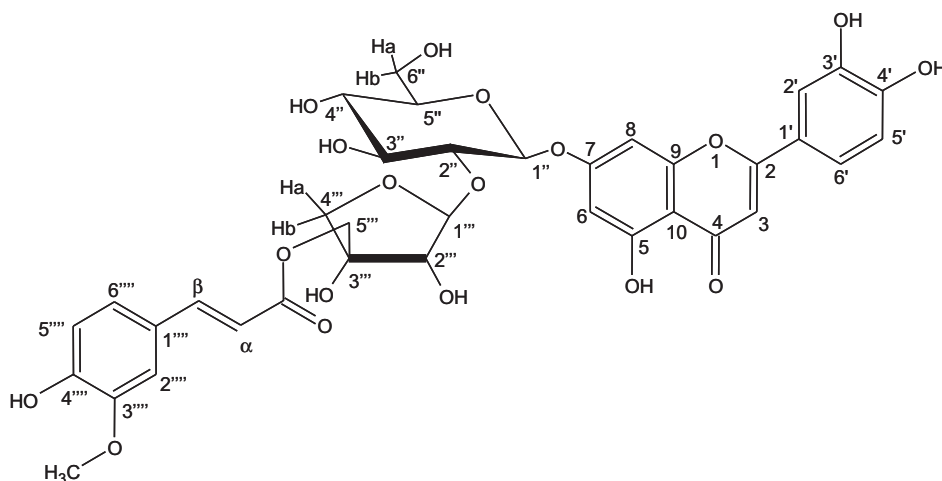


Figure 1. Structure of compound (**1**).

correlation between the hydrogen signal at δ 4.06 (H-5''' apiose) and the carbon signal at δ 166.3 (*E*-feruloyl, C=O) thus evidencing the esterification at this position. The foregoing evidences in combination with the downfield shift of the C-5''' apiofuranosyl (δ 66.6) when compared to a non-acylated analogue (δ 62.4) also supported this conclusion. Therefore, compound (**1**) was identified as luteolin-7-*O*-[2''-*O*-(5'''-*O*-feruloyl)- β -D-apiofuranosyl]- β -D-glucopyranoside (Figure 1).

A few reports were observed describing the presence of flavonoids in the Nyctaginaceae family. Until now only flavonols were related, such as kaempferol and quercetin from the *Bougainvillea glabra*⁶ and *B. spectabilis*.⁷ Being thus, *Neea theifera* is the unique species of Nyctaginaceae, which produces flavones. These data are important because reveal to be able in the future to establish a taxonomic marker in the genus or in this species.

Acknowledgments

We thank Dr. Nivaldo Boralle from Instituto de Química UNESP de Araraquara-SP for recording the NMR spectra, to Dr. Norberto Peporine Lopes from Faculdade de Filosofia Ciências e Letras USP de Ribeirão Preto-SP for HRESIMS analysis, to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for funding and fellowships to D. Rinaldo and M. Sannomiya, to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for grants to W. Vilegas.

Supplementary Information

Supplementary data are available free of charge at <http://jbcbs.sbcq.org.br>, as PDF file.

References

- Durigan, G.; Baitello, J. B.; Franco, G. A. D. C.; de Siqueira, M. F.; *Plantas do Cerrado Paulista : Imagens de uma Paisagem Ameaçada*, Páginas & Letras Editora e Gráfica: São Paulo, 2004.
- Edeoga, H. O.; Ikem, C. I.; *S. Afr. J. Bot.* **2002**, *68*, 382.
- Ahmed, B.; Yu, C. P.; *Phytochemistry* **1992**, *31*, 4382.
- Lami, N.; Kadota, S.; Kikuchi, T.; *Chem. Pharm. Bull.* **1991**, *39*, 1863.
- Lami, N.; Kadota, S.; Kikuchi, T.; Momose, Y.; *Chem. Pharm. Bull.* **1991**, *39*, 1551.
- Heuer, S.; Richter, S.; Metzger, J. W.; Wray, V.; Nimtz, M.; Strack, D.; *Phytochemistry* **1994**, *37*, 761.
- Chang, W. S.; Lee, Y. J.; Lu, F. J.; Chiang, H. C.; *Anti-Cancer Res.* **1993**, *13*, 2165.
- Chang, W. S.; Chang, Y. H.; Lu, F. J.; Chiang, H.; *Anti-Cancer Res.* **1994**, *14*, 501.
- De Feo, V.; Piacente, S.; Pizza, C.; Soria, R. U.; *Biochem. Syst. Ecol.* **1998**, *26*, 251.
- Lavaud, C.; Beauviere, S.; Massiot, G.; Lemenolivier, L.; Bourdy, G.; *Phytochemistry* **1996**, *43*, 189.
- Duke, J.; Vasquez, R.; *Amazonian Ethnobotanical Dictionary*, CRC Press: Boca Raton, 1994.
- Mabry, T. J.; Markham, K. R.; Thomas, M. B.; *The Systematic Identification of Flavonoids*, Springer: New York, 1970.
- Harborne, J. B.; *The Flavonoids: Advances in Research Since 1986*, Chapman and Hall: London, 1996.
- Agrawal, P. K.; *Carbon 13 of Flavonoids*, Elsevier: New York, 1989.
- dos Santos, L.C.; Piacente, S.; Pizza, C.; Toro, R.; Sano, P. T.; Vilegas, W.; *Biochem. Syst. Ecol.* **2002**, *30*, 451.
- Angyal, S. J.; Bodkin, C. L.; Mills, J. A.; Pojer, P. M.; *Aust. J. Chem.* **1977**, *30*, 1259.
- Tronchet, J. M. J.; Tronchet, J.; *Carbohydr. Res.* **1974**, *34*, 263.
- Kitagawa, I.; Sakagami, M.; Hashiuchi, F.; Zhou, J. L.; Yoshikawa, M.; Ren, J.; *Chem. Pharm. Bull.* **1989**, *37*, 551.
- Jung, M. J.; Kang, S. S.; Jung, Y. J.; Choi, J. S.; *Chem. Pharm. Bull.* **2004**, *52*, 1501.
- Mathias, L.; Vieira, I. J. C.; Braz-Filho, R.; Rodrigues-Filho, E. A.; *J. Nat. Prod.* **1998**, *61*, 1158.
- Bashir, A.; Hamburger, M.; Gupta, M. P.; Solis, P. N.; Hostettmann, K.; *Phytochemistry* **1991**, *30*, 3781.

Received: December 20, 2006

Web Release Date: September 4, 2007

FAPESP helped in meeting the publication costs of this article.

New Flavone from the Leaves of *Neea theifera* (Nyctaginaceae)

Daniel Rinaldo, Clenilson M. Rodrigues, Juliana Rodrigues, Miriam Sannomiya,
Lourdes C. dos Santos and Wagner Vilegas*

Instituto de Química, Universidade Estadual Paulista, CP 355, 14801-900 Araraquara-SP, Brazil

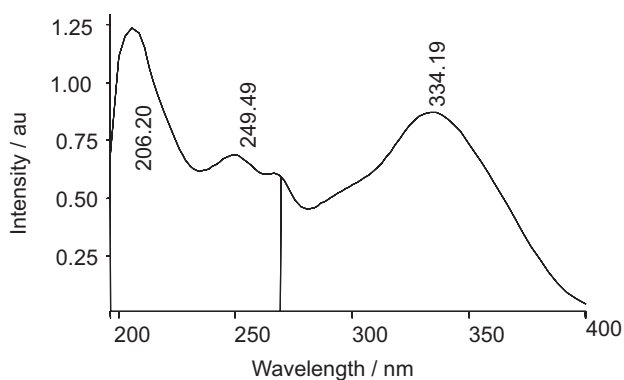


Figure S1. UV spectrum of (1) (MeOH).

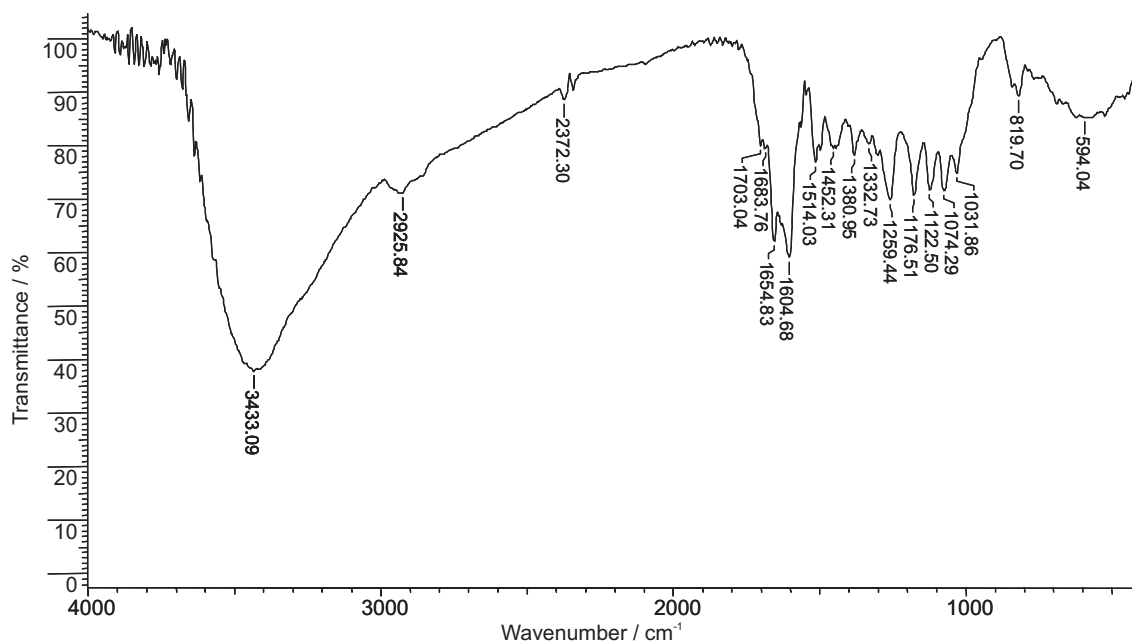
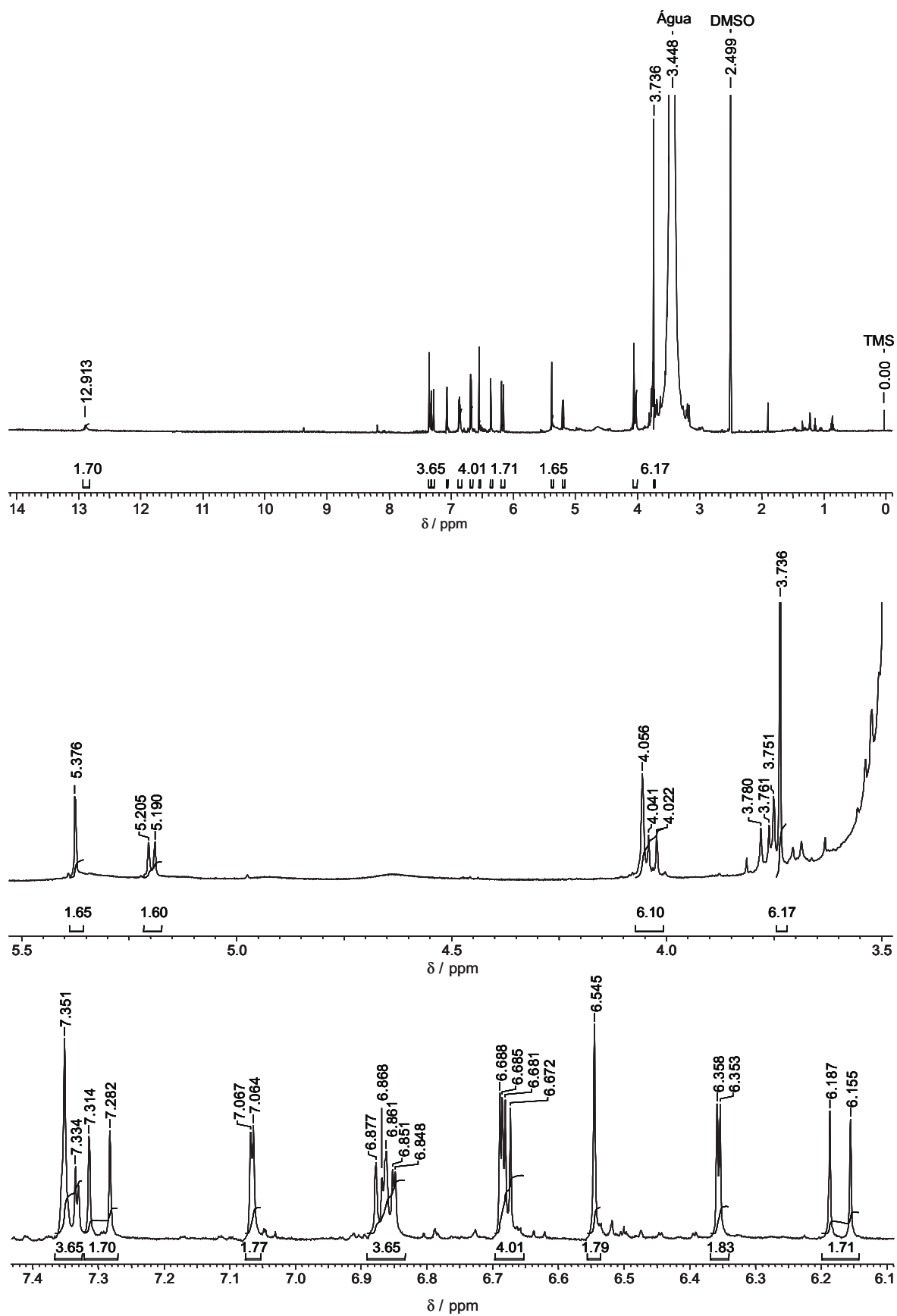


Figure S2. IR spectrum of (1) (KBr disk).

*e-mail: vilegasw@gmail.com

Figure S3. ^1H NMR spectrum of (1) ($\text{DMSO-}d_6$, 11.7 T, TMS, ppm).

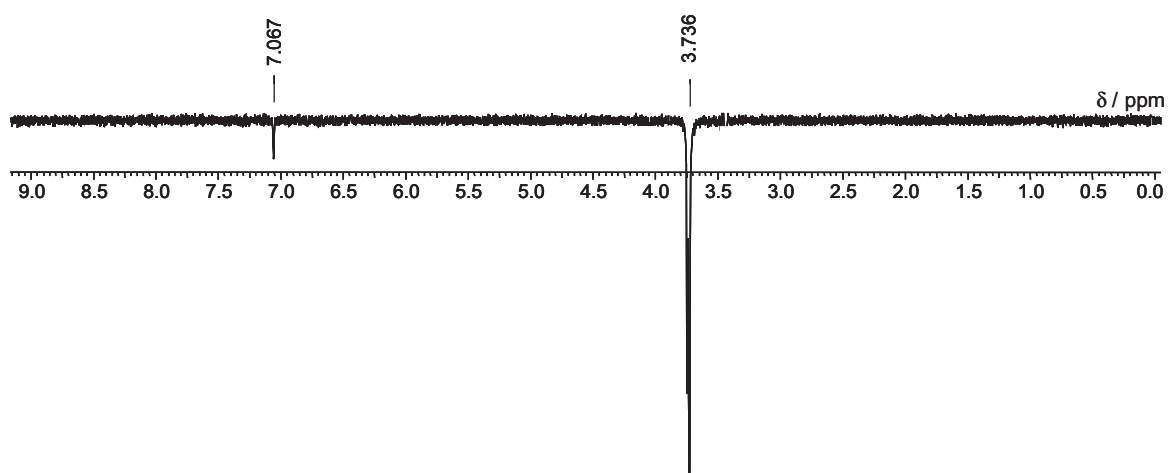


Figure S4. ¹H NMR 1D-NOESY of (I) (DMSO-*d*₆, 11.7 T, ppm).

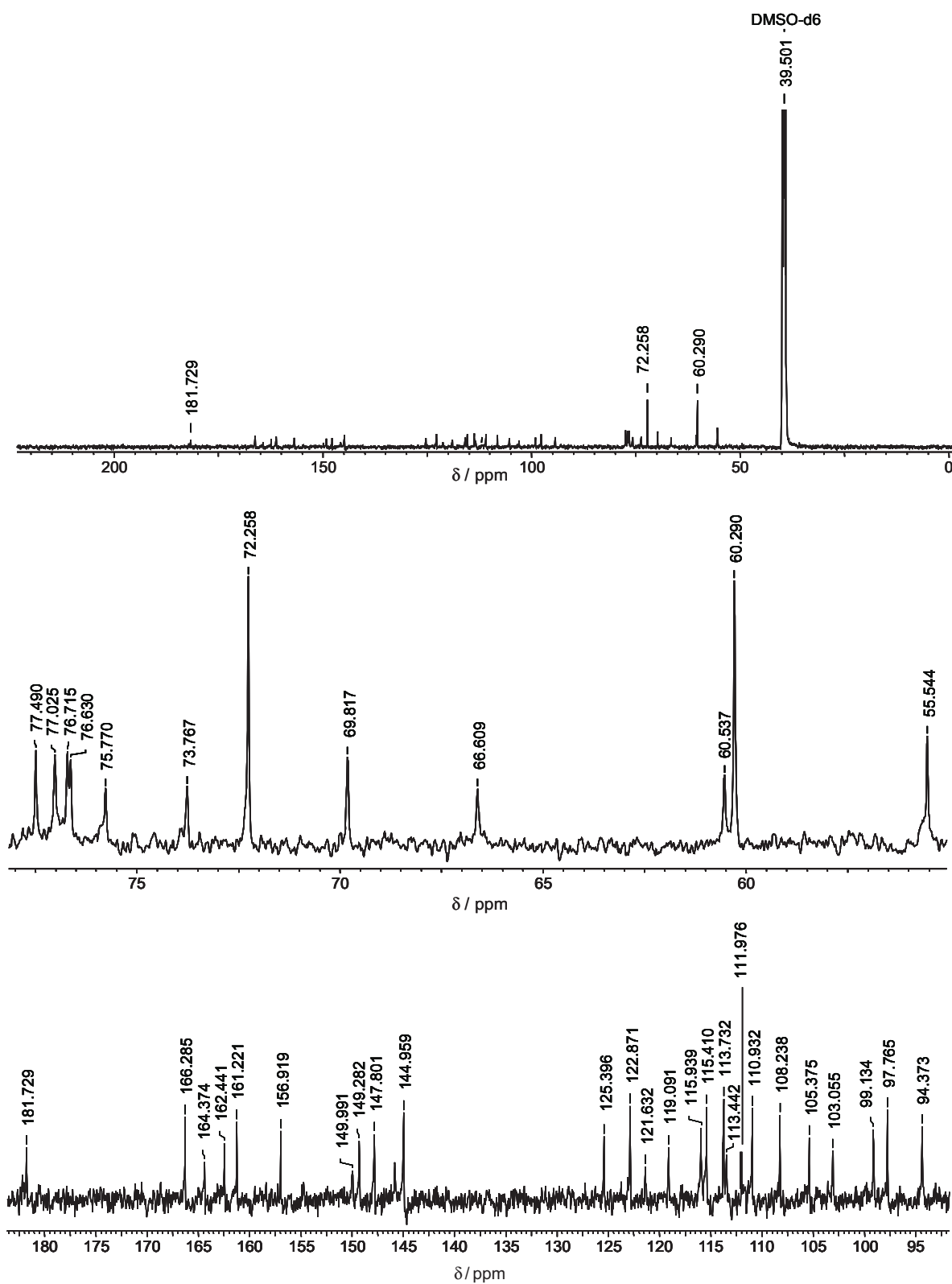


Figure S5. ^{13}C NMR spectrum of (I) (DMSO- d_6 , 11.7 T, ppm).

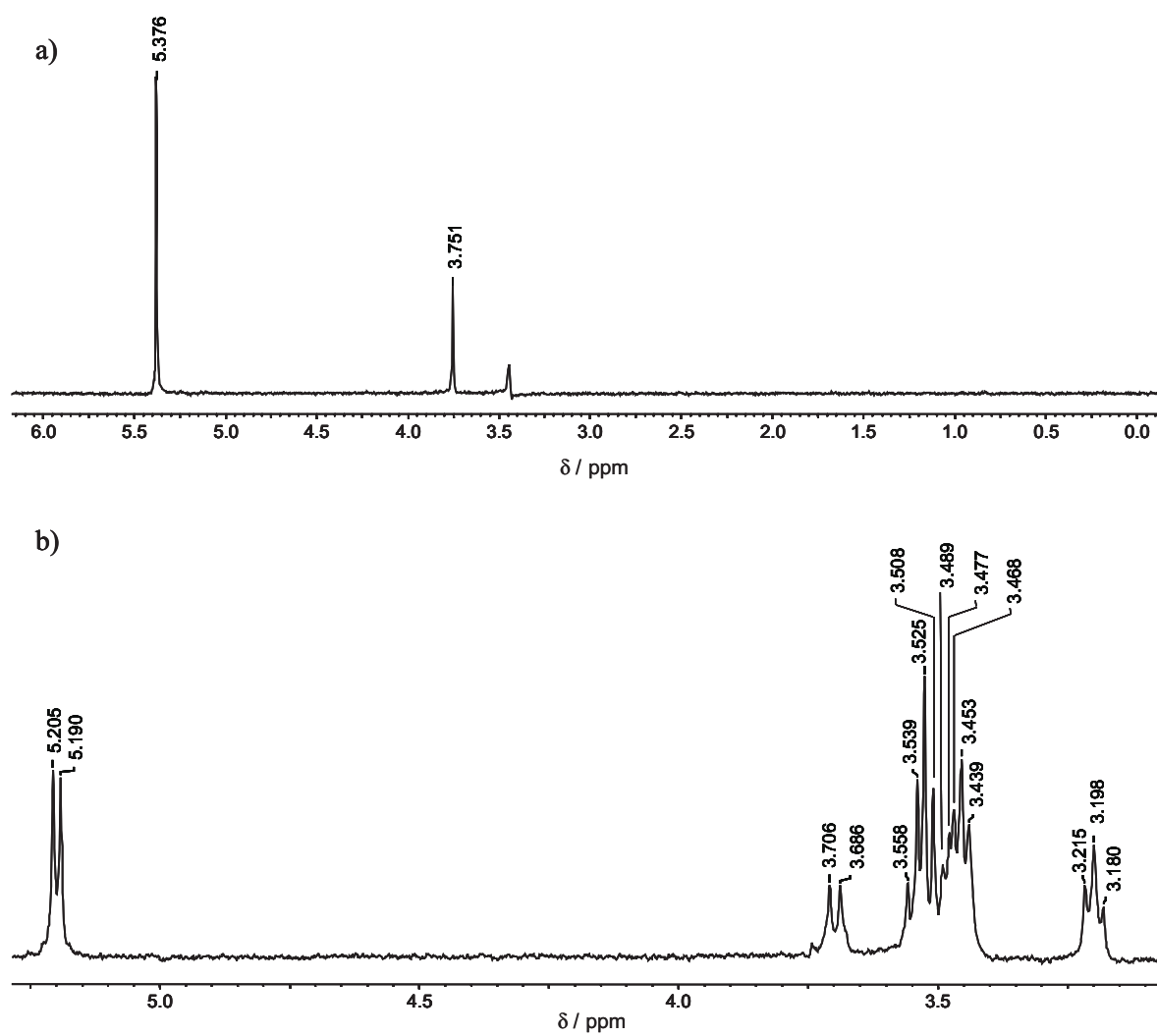


Figure S6. TOCSY spectrum of (I) (DMSO- d_6 , 11.7 T, ppm): a) Apiose; b) Glucose.

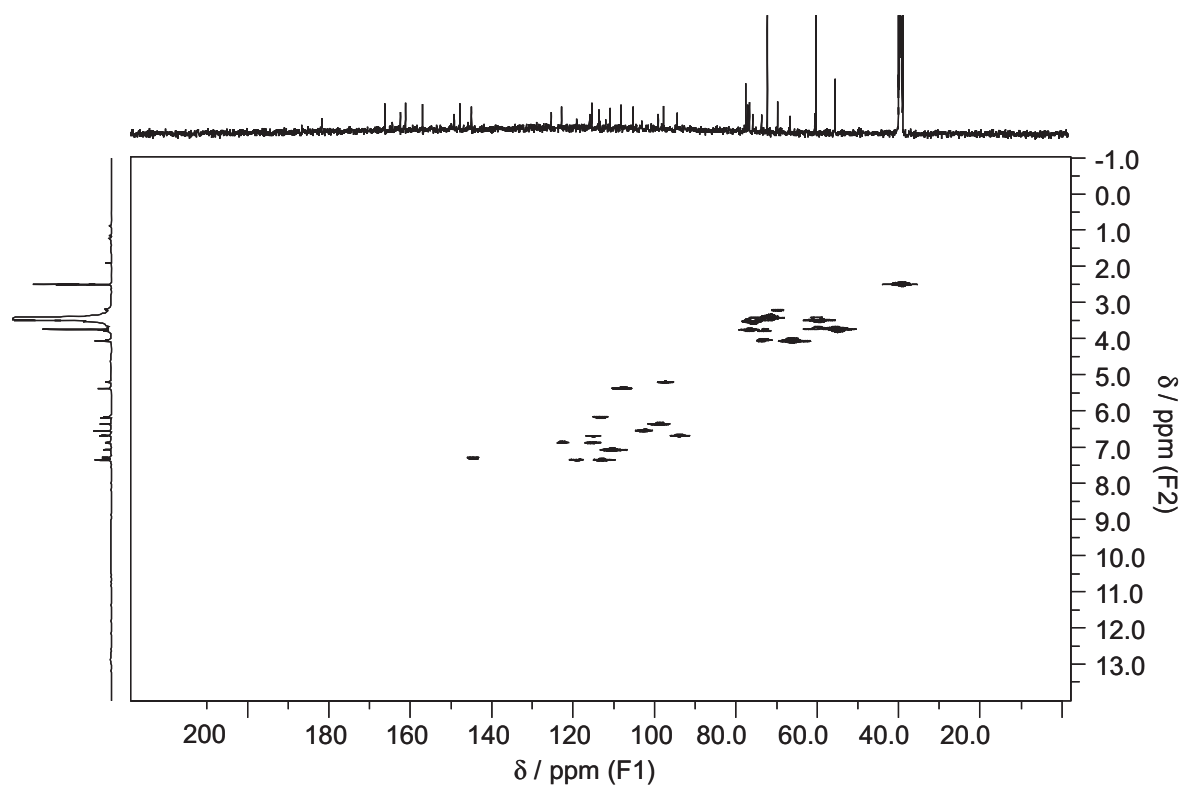


Figure S7. gHMBC spectrum of (1) (DMSO-*d*₆, 11.7 T, TMS, ppm).

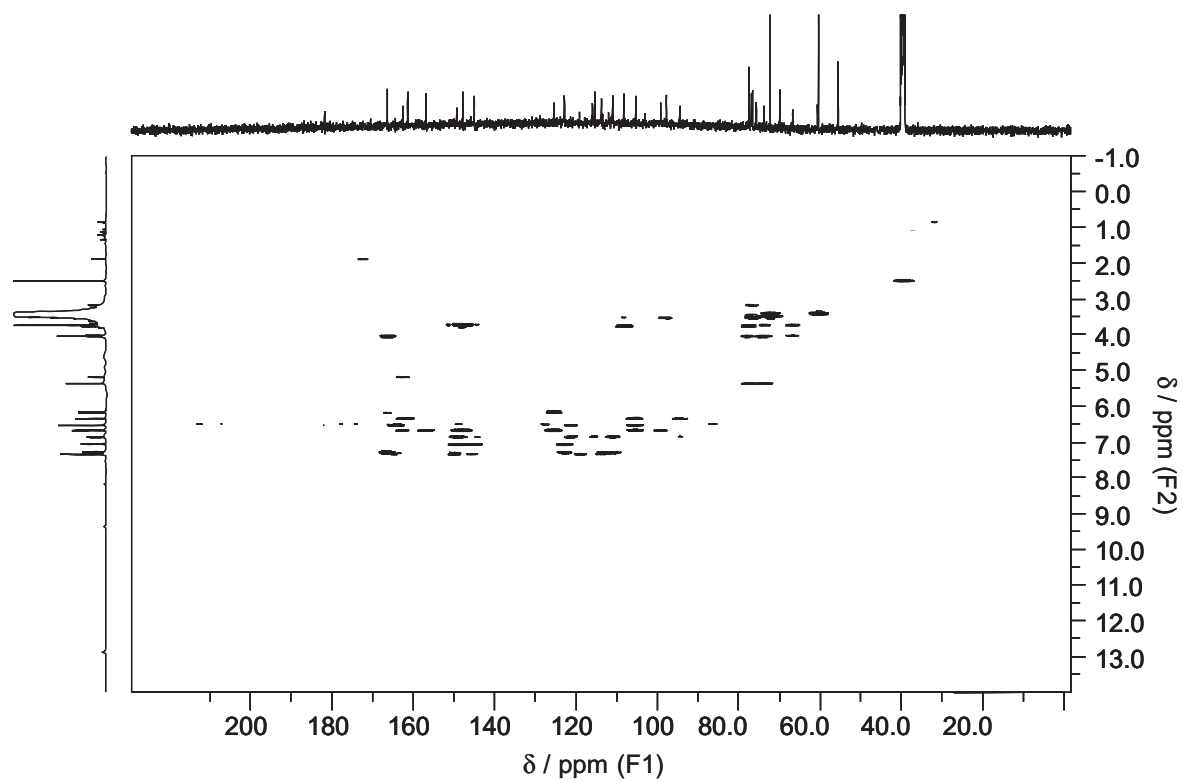


Figure S8. gHMBC spectrum of (1) (DMSO-*d*₆, 11.7 T, TMS, ppm).

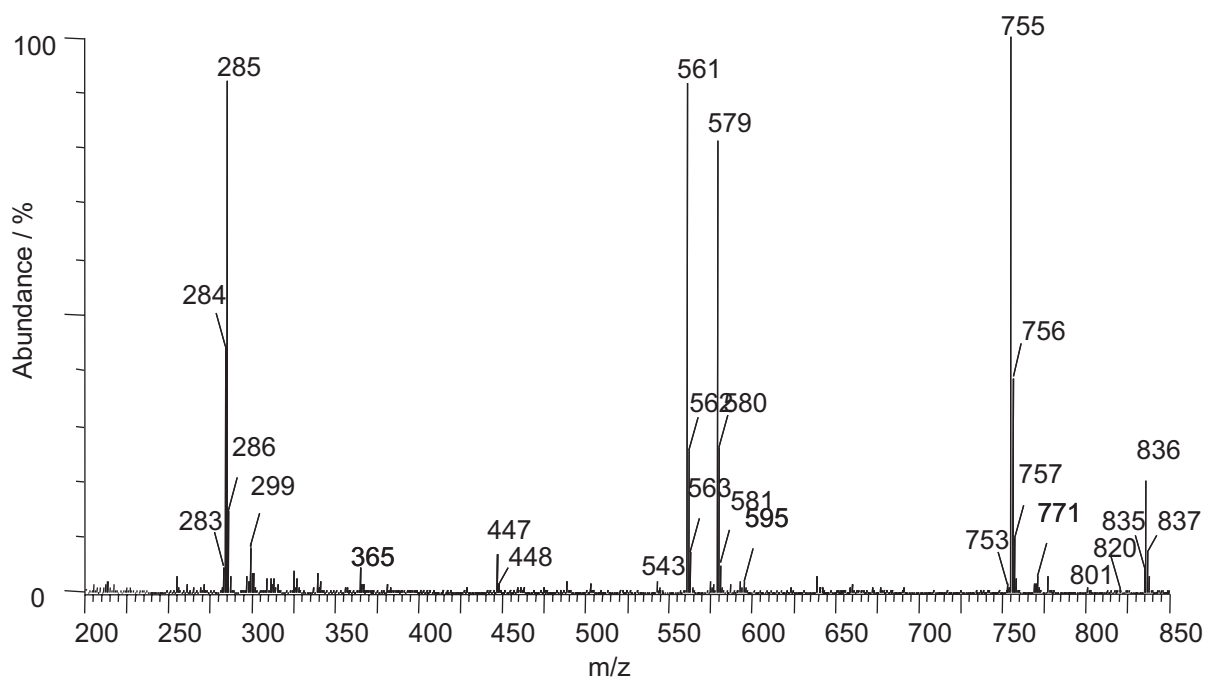


Figure S9. ESI-MS spectrum of (1) (negative mode, 70 V).

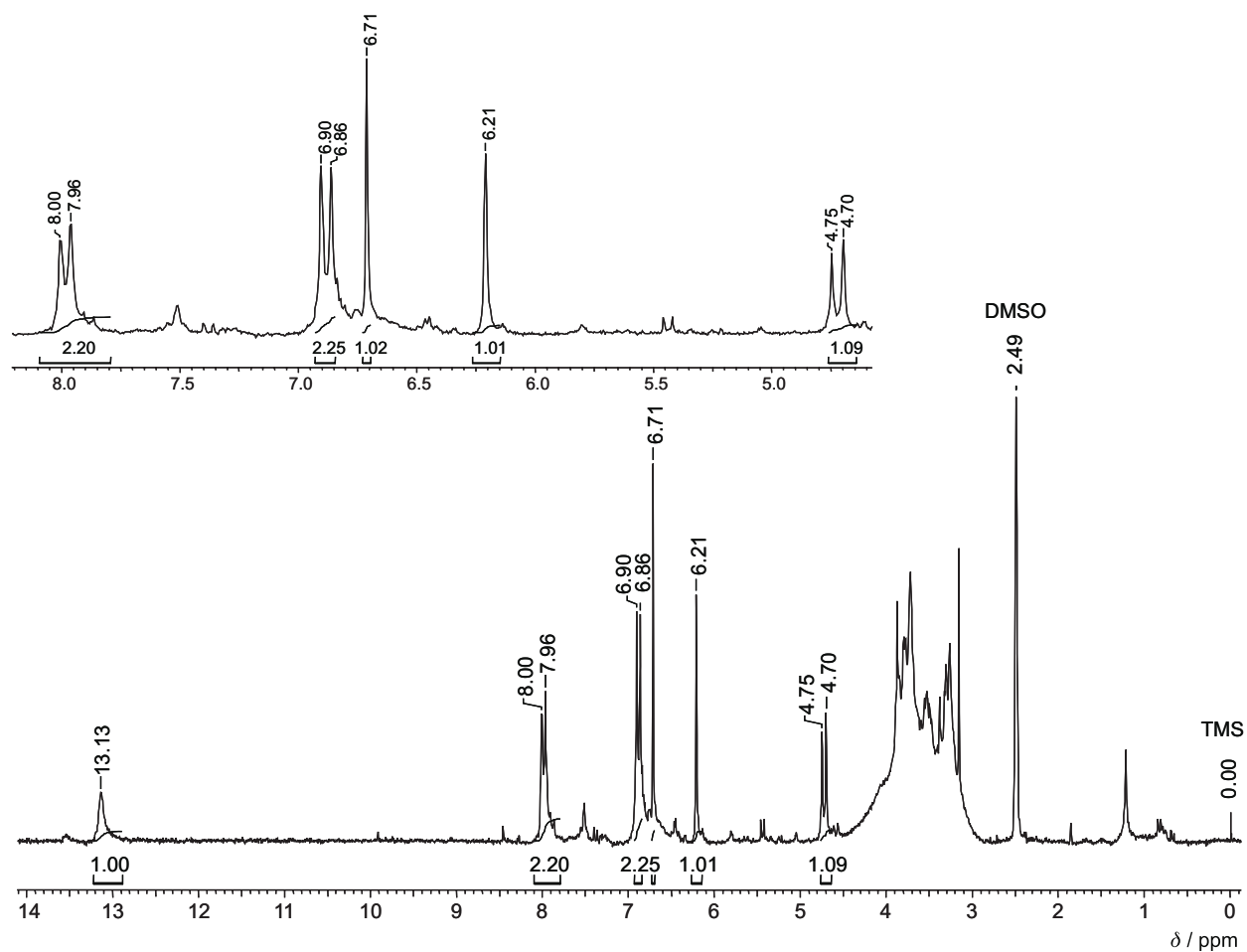


Figure S10. ^1H NMR spectrum of vitexin ($\text{DMSO-}d_6$, 4.7 T, TMS, ppm).

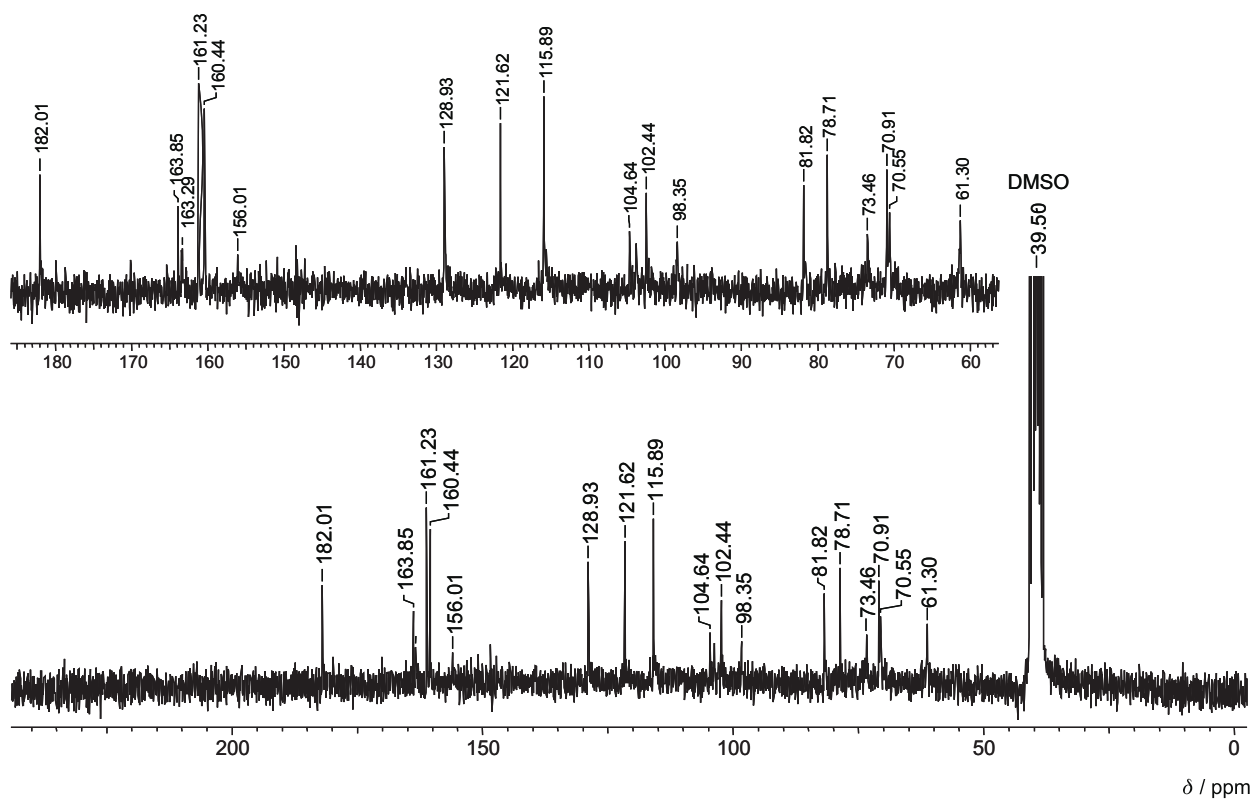


Figure S11. ^{13}C NMR spectrum of vitexin (DMSO-d_6 , 4.7 T, ppm).

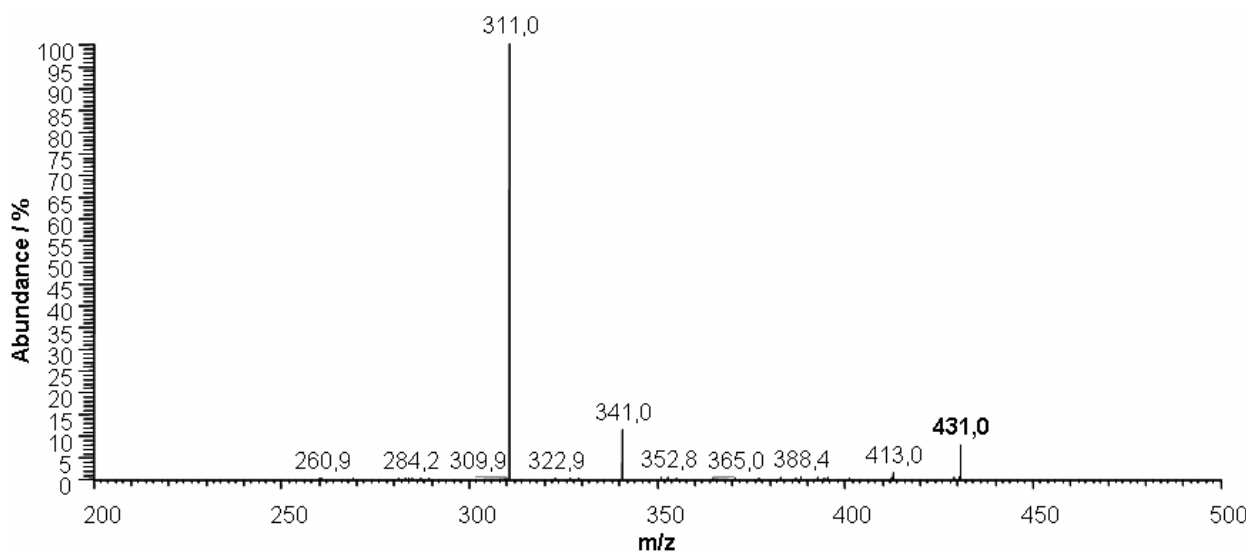


Figure S12. ESI-MS spectrum of vitexin (negative mode, 70 V).

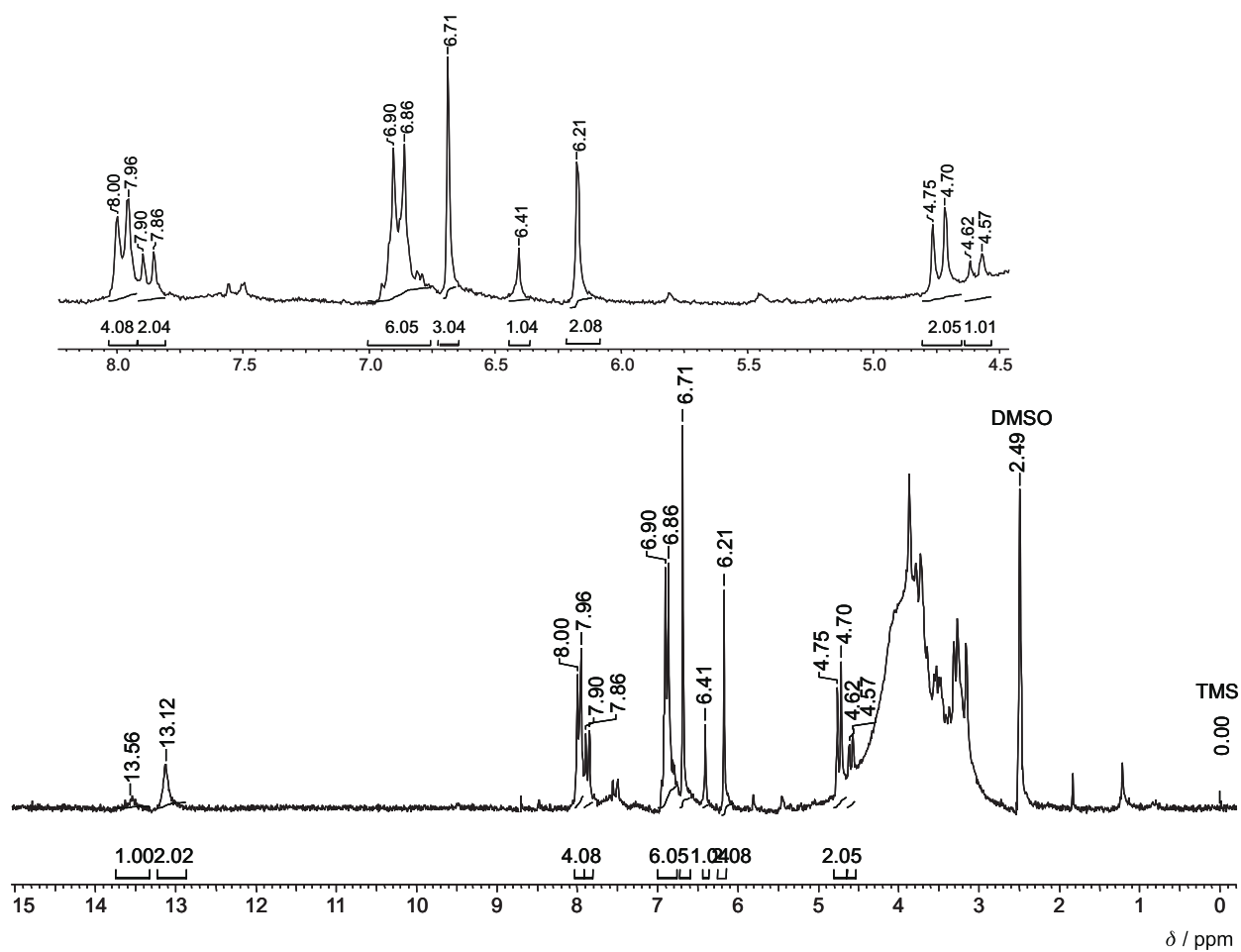


Figure S13. ^1H NMR spectrum of the mixture of isovitexin and vitexin ($\text{DMSO-}d_6$, 4.7 T, TMS, ppm).

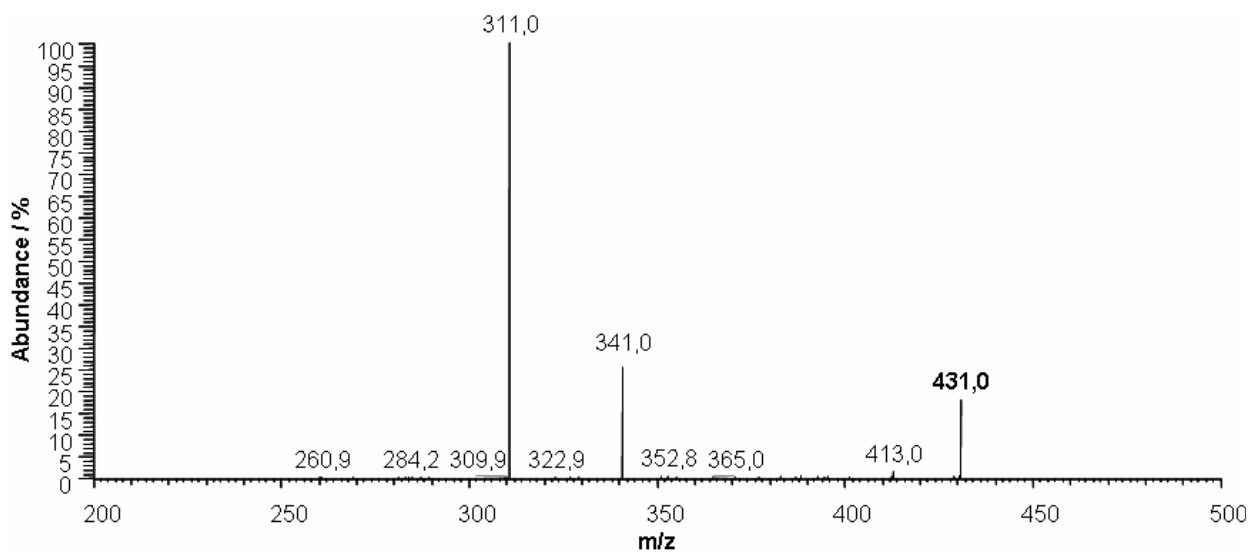


Figure S14. ESI-MS spectrum of the mixture of isovitexin and vitexin (negative mode, 70 V).

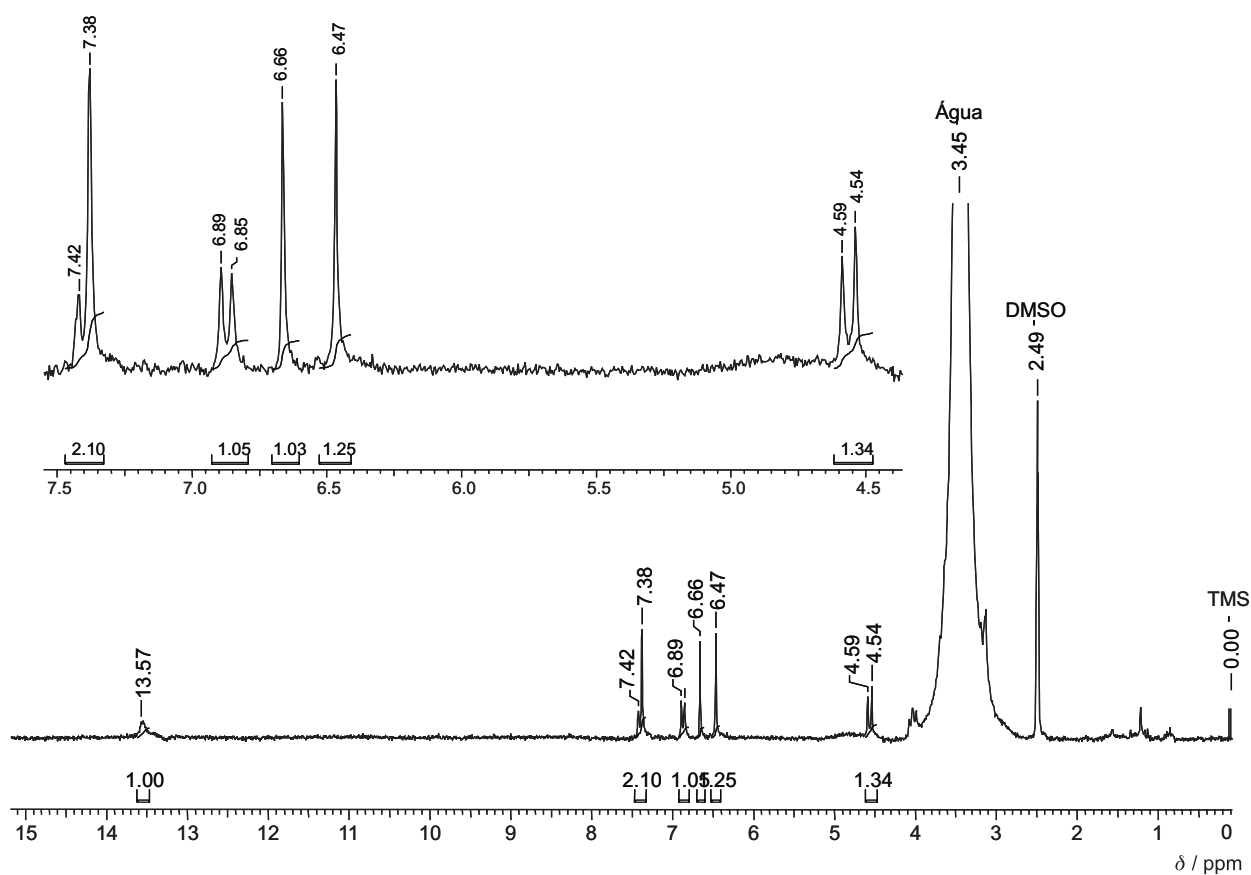


Figure S15. ^1H NMR spectrum of isoorientin ($\text{DMSO-}d_6$, 4.7 T, TMS, ppm).

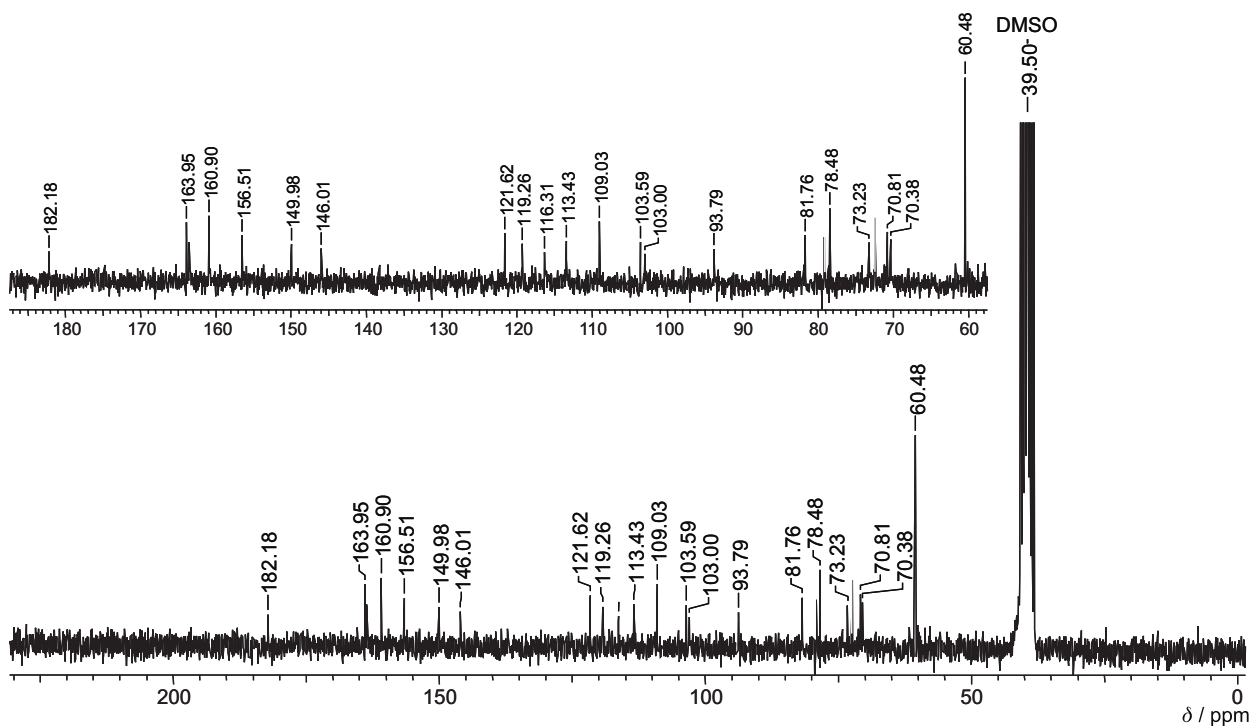


Figure S16. ^{13}C NMR spectrum of isoorientin ($\text{DMSO-}d_6$, 4.7 T, ppm).

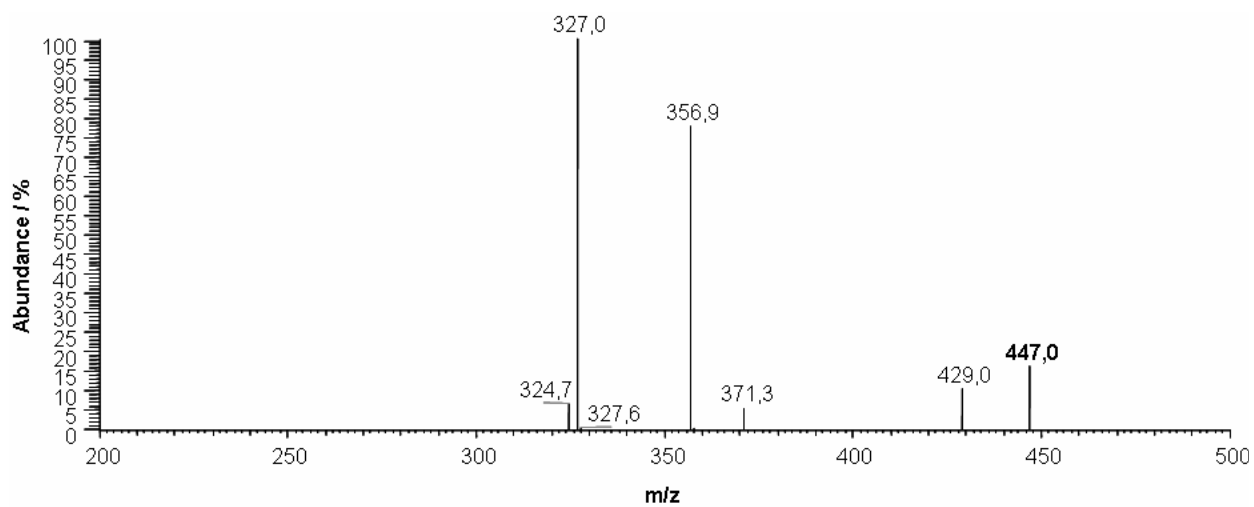


Figure S17. ESI-MS spectrum of isoorientin (negative mode, 70 V).

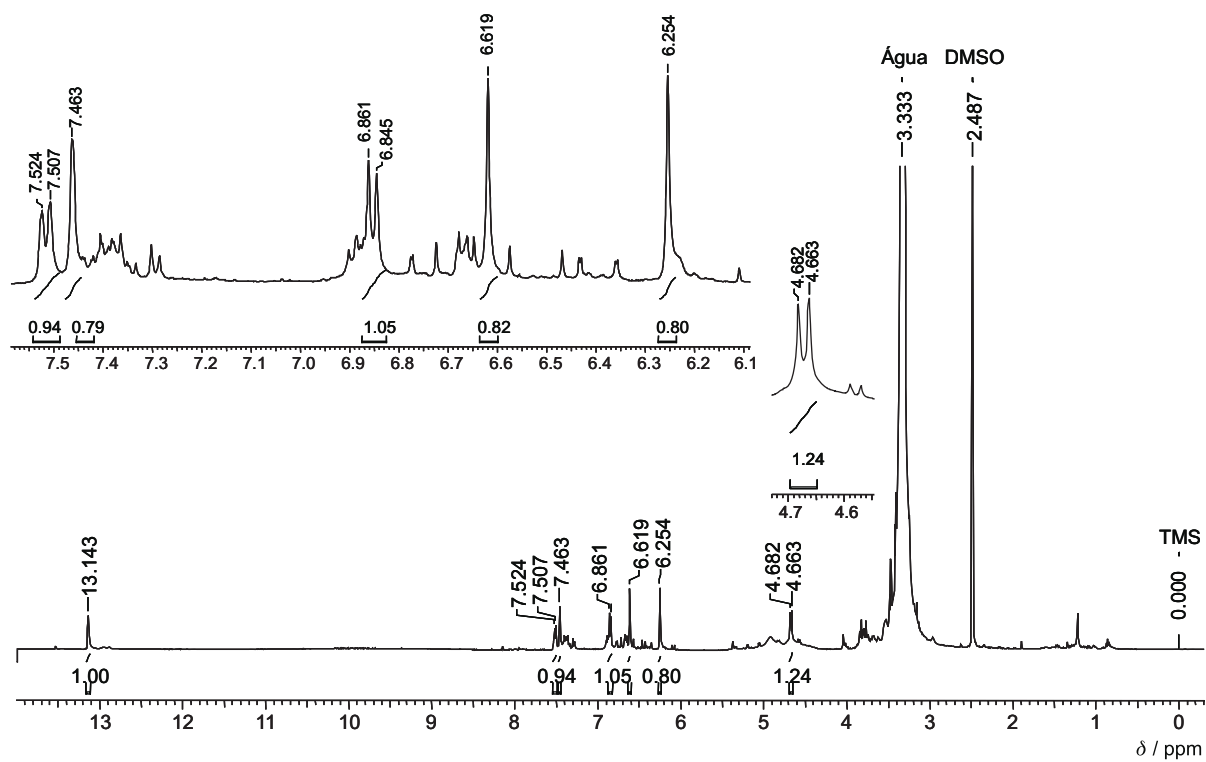


Figure S18. ^1H NMR spectrum of orientin ($\text{DMSO-}d_6$, 4.7 T, TMS, ppm).

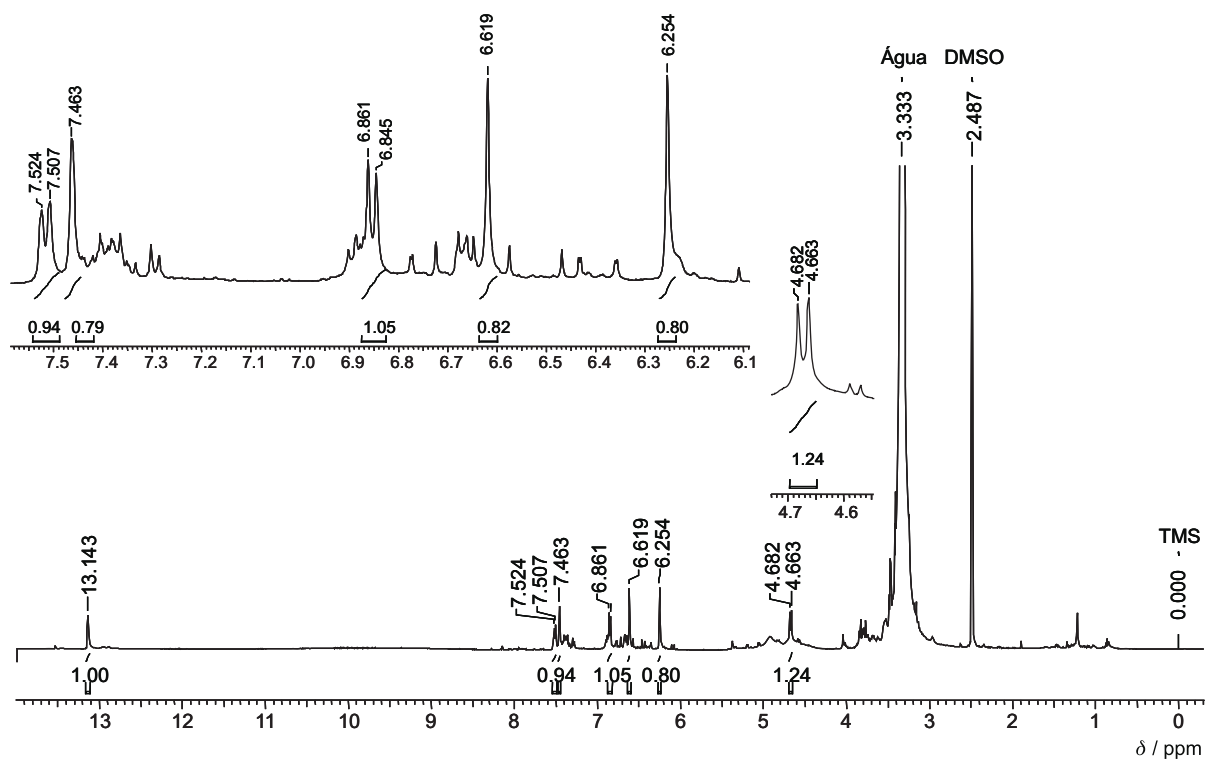


Figure S19. ^{13}C NMR spectrum of orientin (DMSO- d_6 , 4.7 T, ppm).

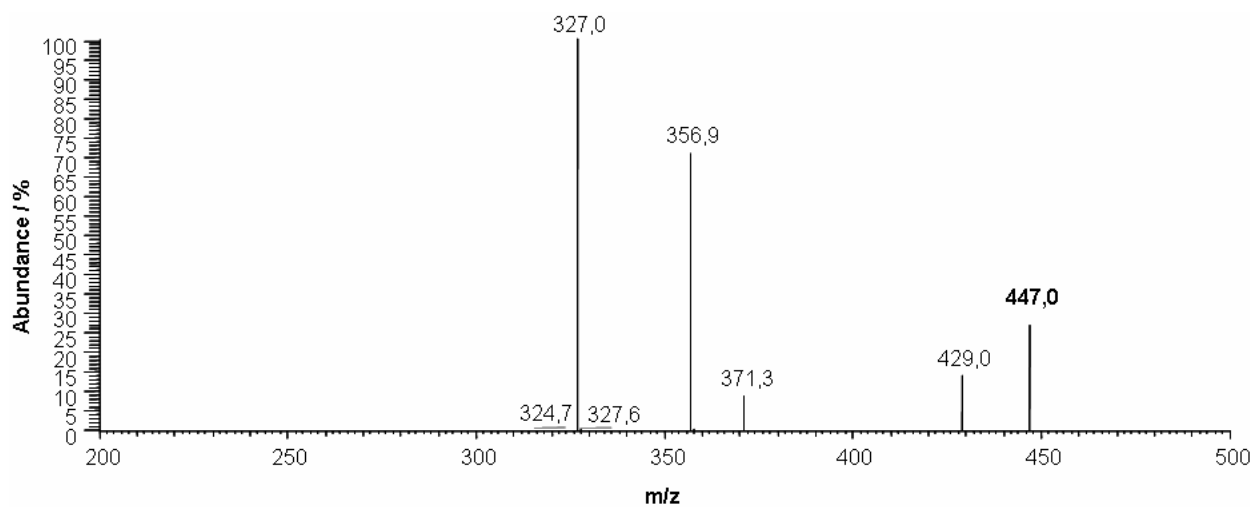


Figure S20. ESI-MS spectrum of orientin (negative mode, 70 V).

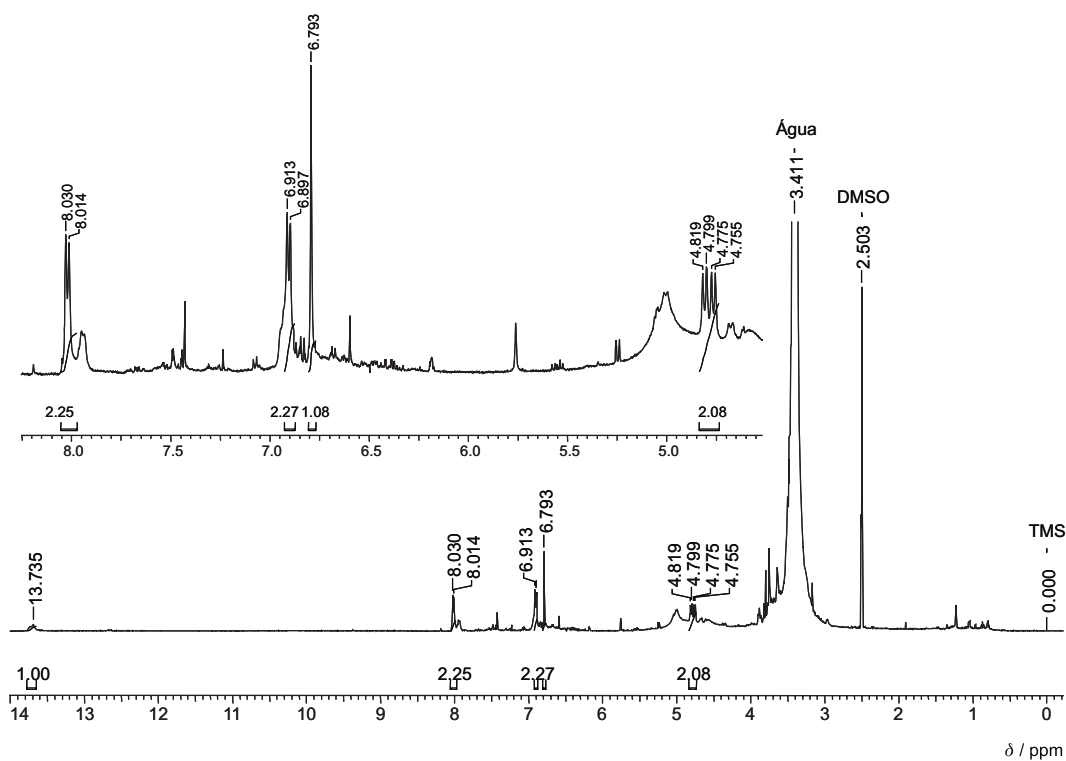


Figure S21. ^1H NMR spectrum of vicenin-2 ($\text{DMSO-}d_6$, 11.7 T, TMS, ppm).

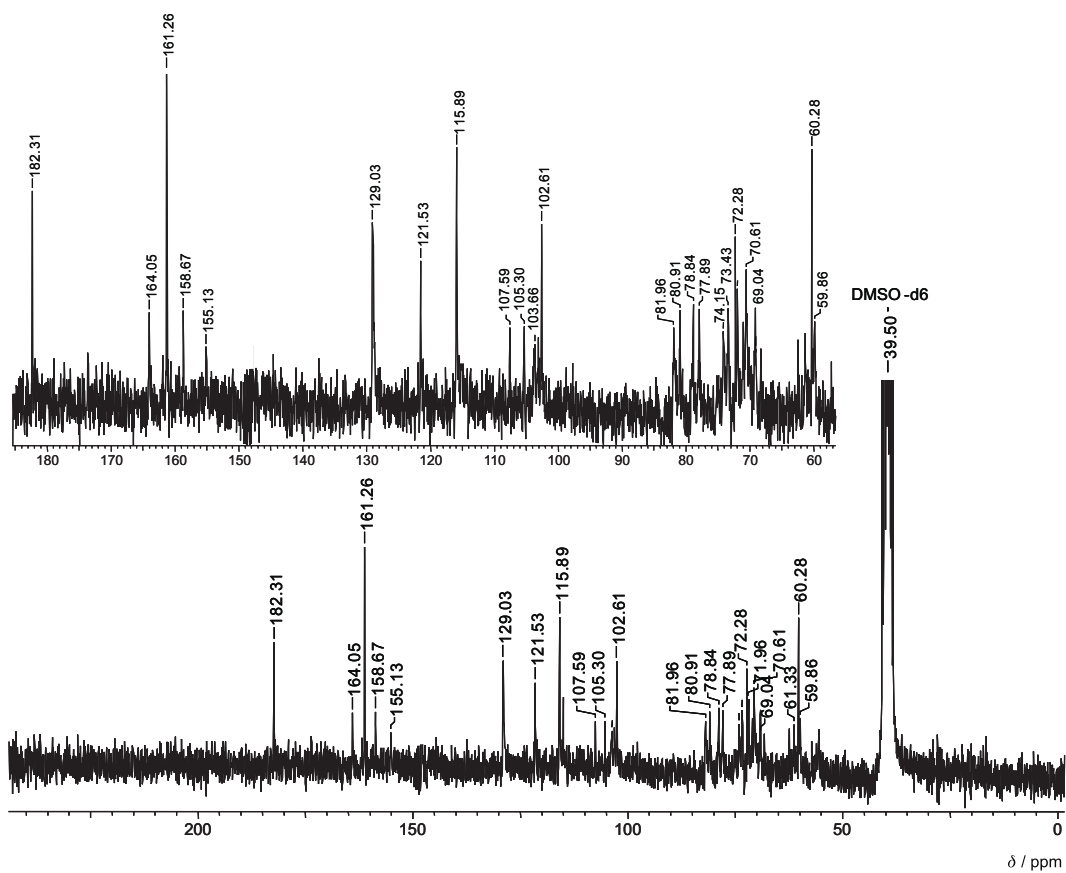


Figure S22. ^{13}C NMR spectrum of vicenin-2 ($\text{DMSO-}d_6$, 11.7 T, TMS, ppm).

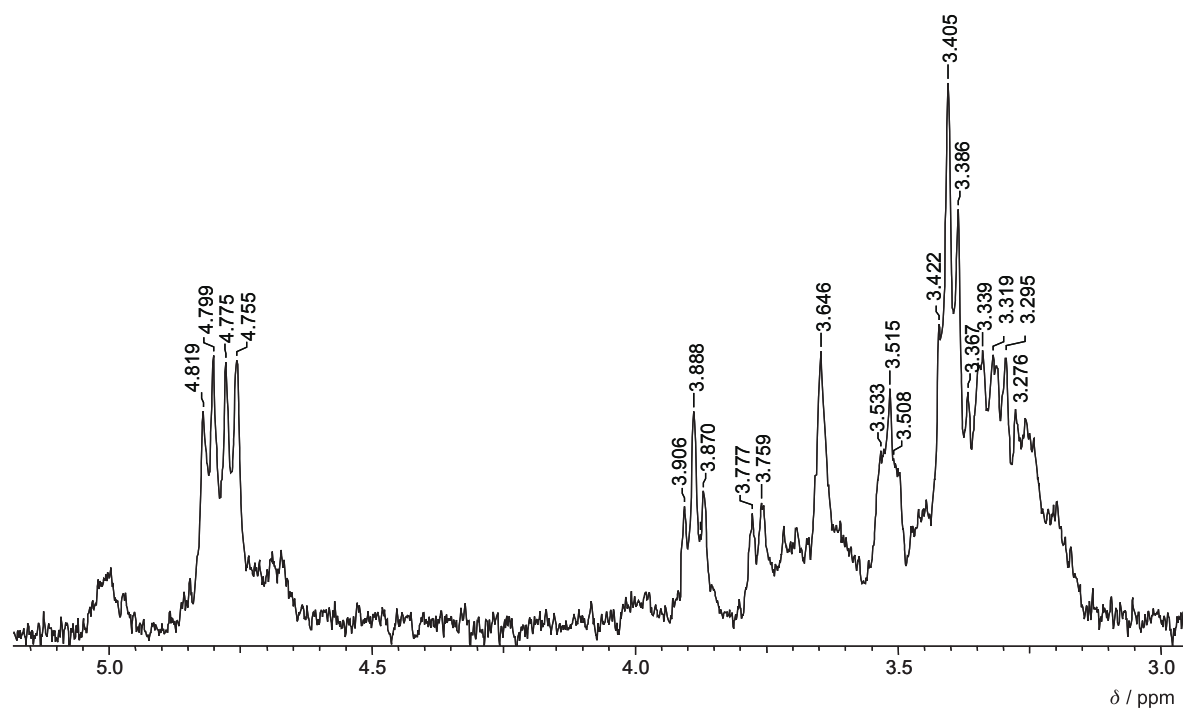


Figure S23. TOCSY spectrum of vicenin-2 (DMSO- d_6 , 11.7 T, ppm, irradiation of δ 4.809).

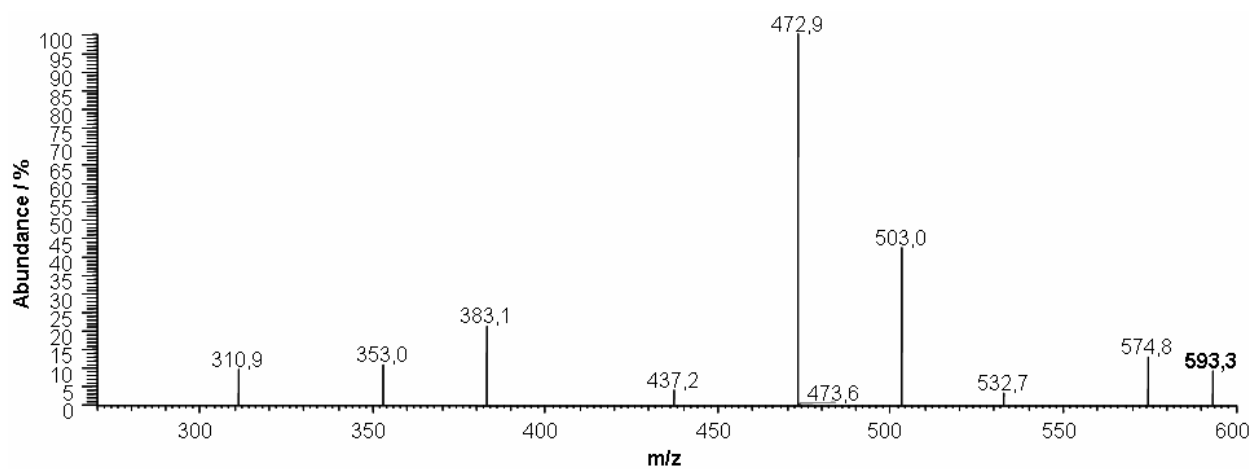


Figure S24. ESI-MS spectrum of vicenin-2 (negative mode, 70 V).

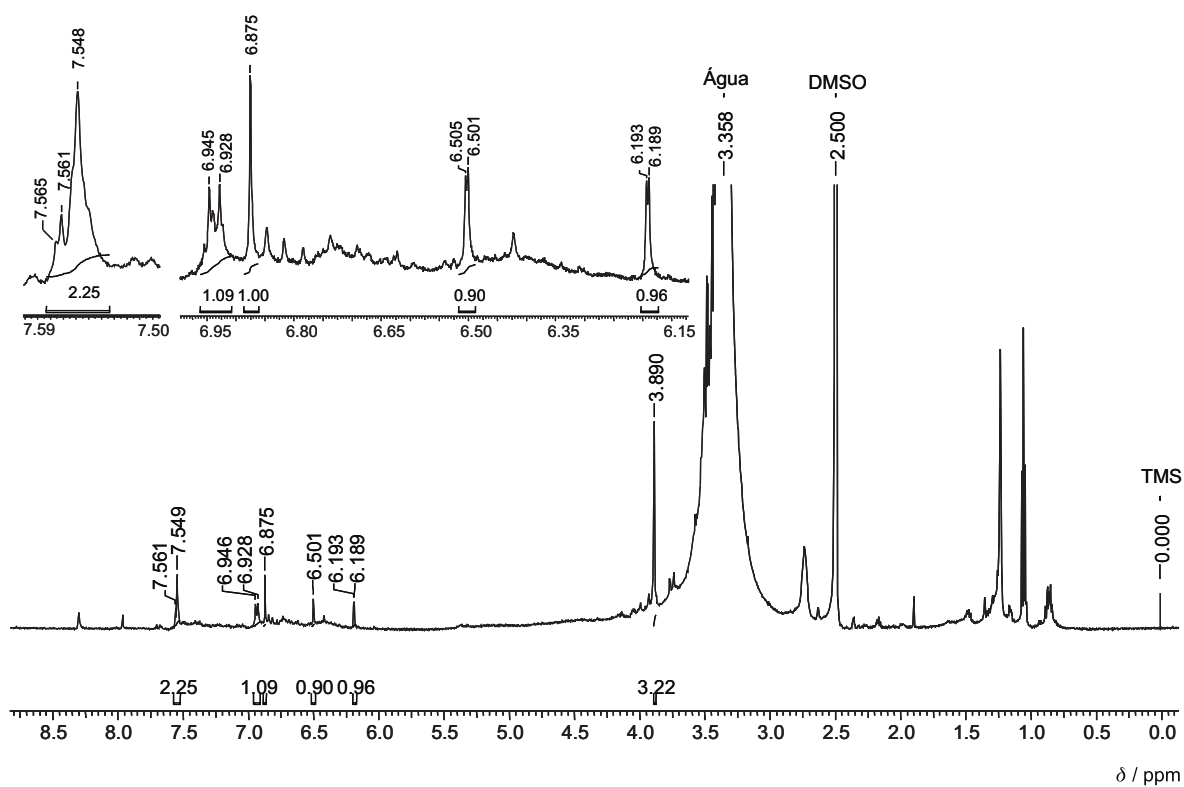


Figure S25. ¹H NMR spectrum of chrysoeriol (DMSO-*d*₆, 11.7 T, ppm).

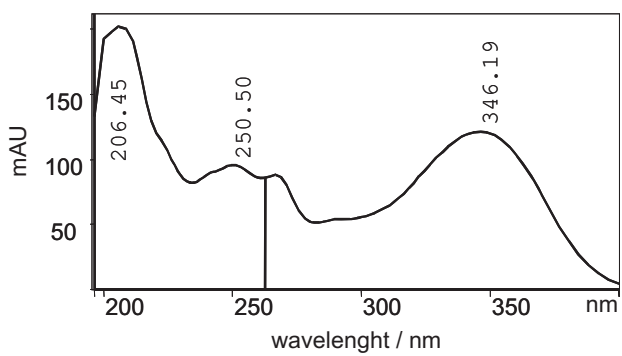


Figure S26. UV spectrum of chrysoeriol (MeOH).

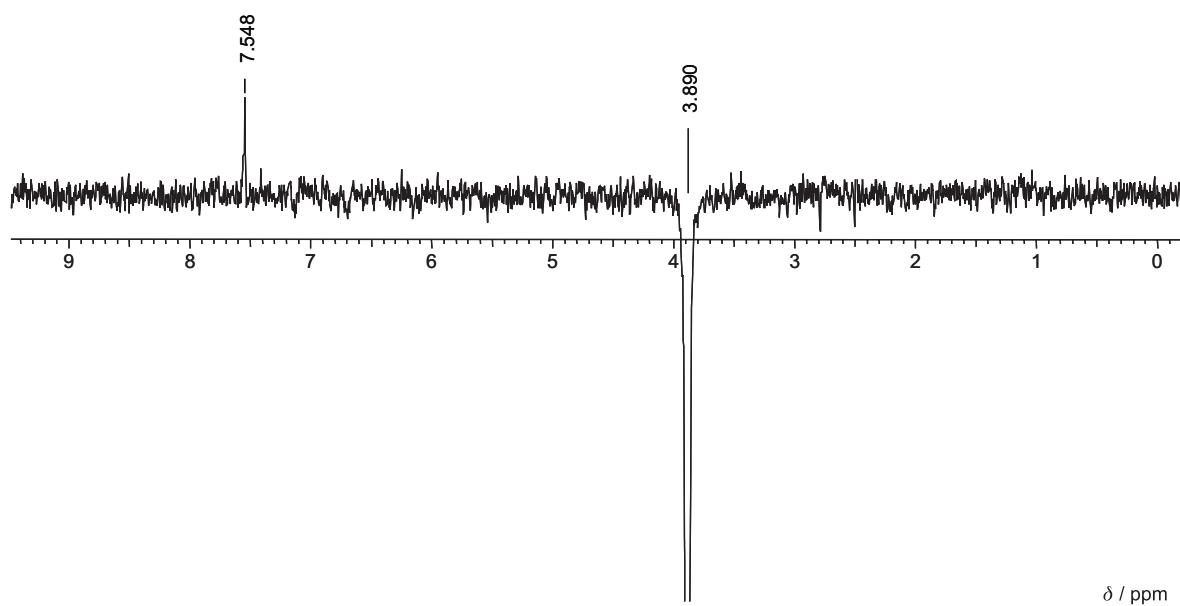


Figure S27. ^1H NMR 1D-NOESY of chrysoeriol ($\text{DMSO-}d_6$, 11.7 T, ppm, irradiation of δ 3.890).

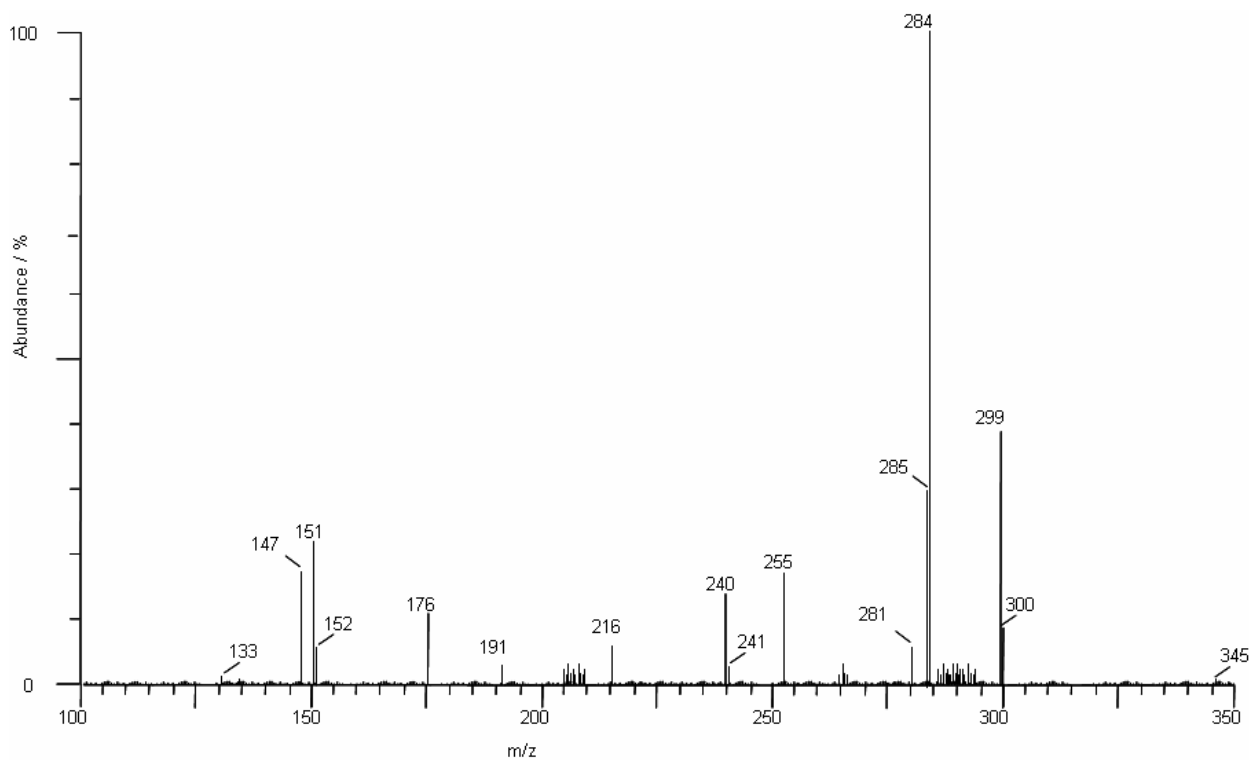


Figure S28. ESI-MS spectrum of chrysoeriol (negative mode, 70 V).

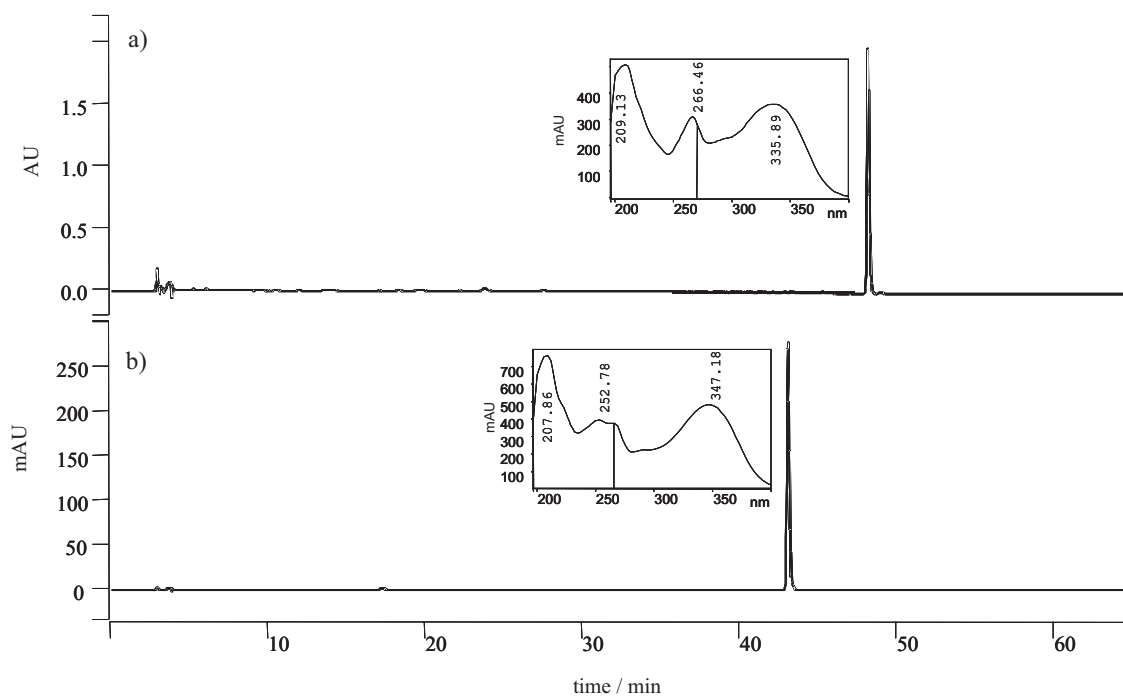


Figure S29. Chromatograms of the co-injections of a) apigenin isolated + apigenin Sigma® (1:1 m/m) and b) luteolin isolated + luteolin Sigma® (1:1 m/m) (C18, 250 × 4.60 mm i. d. × 5 µm). A binary gradient elution system with solvent A (0.05% TFA in H₂O) and solvent B (0.05% TFA in CH₃CN) was used, with linear gradient starting from 68:32 (A:B) at 20 min and then changed to 75:35 (A:B) for 40 min. The flow-rate was 1.0 mL min⁻¹. λ: 254 nm.