

Supplementary Material

Laricitrin 3-Rutinoside from *Ginkgo biloba* Fruits Protects Damage in TNF- α -Stimulated Normal Human Dermal Fibroblasts

Sullim Lee ^{1,†}, Yea Jung Choi ^{2,†}, Chen Huo ^{3,†}, Akida Alishir ³, Ki Sung Kang ², Il-Ho Park ⁴, Taesu Jang ^{5,*} and Ki Hyun Kim ^{3,*}

¹ Department of Life Science, College of Bio-Nano Technology, Gachon University, Seongnam 13120, Republic of Korea; sullimlee@gachon.ac.kr (S.L.)

² College of Korean Medicine, Gachon University, Seongnam 13120, Republic of Korea; domdada22@gachon.ac.kr (Y.J.C.); kkang@gachon.ac.kr (K.S.K.)

³ School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea; huochen_0213@163.com (C.H.); akida.alishir@gmail.com (A.A.)

⁴ College of Pharmacy, Sahmyook University, 815, Hwarang-ro, Nowon-gu, Seoul 01795, Republic of Korea; parkilho@syu.ac.kr (I.H.P.)

⁵ Health Administration, Dankook University, Cheonan 31116, Republic of Korea; jangts@dankook.ac.kr (T.J.)

* Correspondence: jangts@dankook.ac.kr (T.J.); khkim83@skku.edu (K.H.K.); Tel.: +82-41-550-1476 (T.J.); +82-31-290-7700 (K.H.K.)

[†] These authors contributed equally to this work.

Figure S1. ^1H -NMR (CD_3OD , 850 MHz) spectrum of (<i>E</i>)-coniferin (1).....	4
Figure S2. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 1.	5
Figure S3. ^1H -NMR (CD_3OD , 850 MHz) spectrum of syringin (2).	6
Figure S4. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 2.	7
Figure S5 ^1H -NMR (CD_3OD , 850 MHz) spectrum of 4-hydroxybenzoic acid 4- <i>O</i> - β -D-glucopyranoside (3).	8
Figure S6. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 3.	9
Figure S7. ^1H -NMR (CD_3OD , 850 MHz) spectrum of vanillic acid 4- <i>O</i> - β -D-glucopyranoside (4).	10
Figure S8. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for 4.	11
Figure S9. ^1H -NMR (CD_3OD , 850 MHz) spectrum of glucosyringic acid (5).	12
Figure S10. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for 5.	13
Figure S11. ^1H -NMR (CD_3OD , 850 MHz) spectrum of (<i>E</i>)-ferulic acid 4- <i>O</i> - β -D-glucoside (6).	14
Figure S12. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for 6.	15
Figure S13. ^1H -NMR (CD_3OD , 850 MHz) spectrum of (<i>E</i>)-sinapic acid 4- <i>O</i> - β -D-glucopyranoside (7).	16
Figure S14. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for 7.	17
Figure S15. ^1H -NMR (CD_3OD , 850 MHz) spectrum of ginkgotoxin-5-glucoside (8).	18
Figure S16. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for 8.	19
Figure S17. ^1H -NMR (CD_3OD , 850 MHz) spectrum of ginkgopanoside (9).	20
Figure S18. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 9.	21

Figure S19. ¹ H-NMR (CD ₃ OD, 850 MHz) spectrum of (Z)-4-coumaric acid 4-O-β-D-glucopyranoside (10).	22
Figure S20. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 10	23
Figure S21. ¹ H-NMR (CD ₃ OD, 850 MHz) spectrum of (1' <i>R</i> ,2' <i>S</i> ,5' <i>R</i> ,8' <i>S</i> ,2' <i>Z</i> ,4' <i>E</i>)-dihydrophaseic acid 3'-O-β-D-glucopyranoside (11).	24
Figure S22. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 11	25
Figure S23. ¹ H-NMR (CD ₃ OD, 850 MHz) spectrum of eucomic acid (12).	26
Figure S24. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 12	27
Figure S25. ¹ H-NMR (CD ₃ OD, 850 MHz) spectrum of rutin (13).	28
Figure S26. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 13	29
Figure S27. ¹ H-NMR (CD ₃ OD, 850 MHz) spectrum of laricitrin 3-rutinoside (14). ..	30
Figure S28. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 14	31
Figure S29. Effect of the extract on NHDF viability	32
Figure S30. Effects of the extract on TNF-α-induced intercellular reactive oxygen species (ROS) generation	33
Figure S31. Effect of the extract on MMP-1 and COL1A1 protein expression in tumor necrosis factor-α (TNF-α)-stimulated normal human dermal fibroblasts (NHDFs)	34

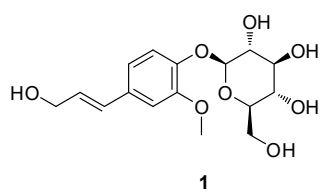
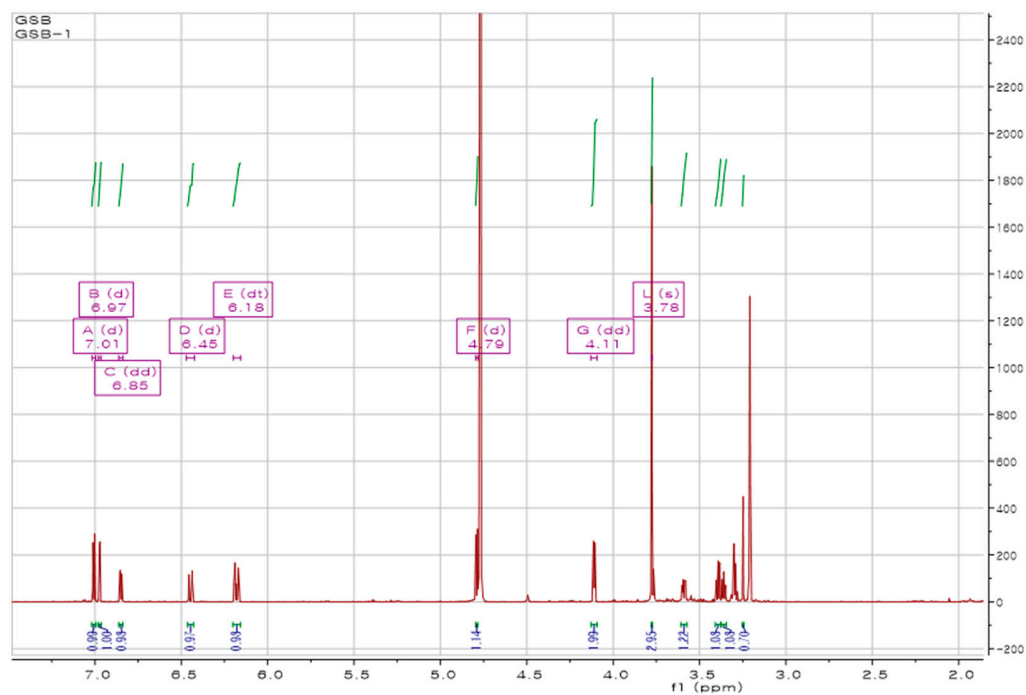


Figure S1. ^1H -NMR (CD_3OD , 850 MHz) spectrum of (*E*)-coniferin (**1**).

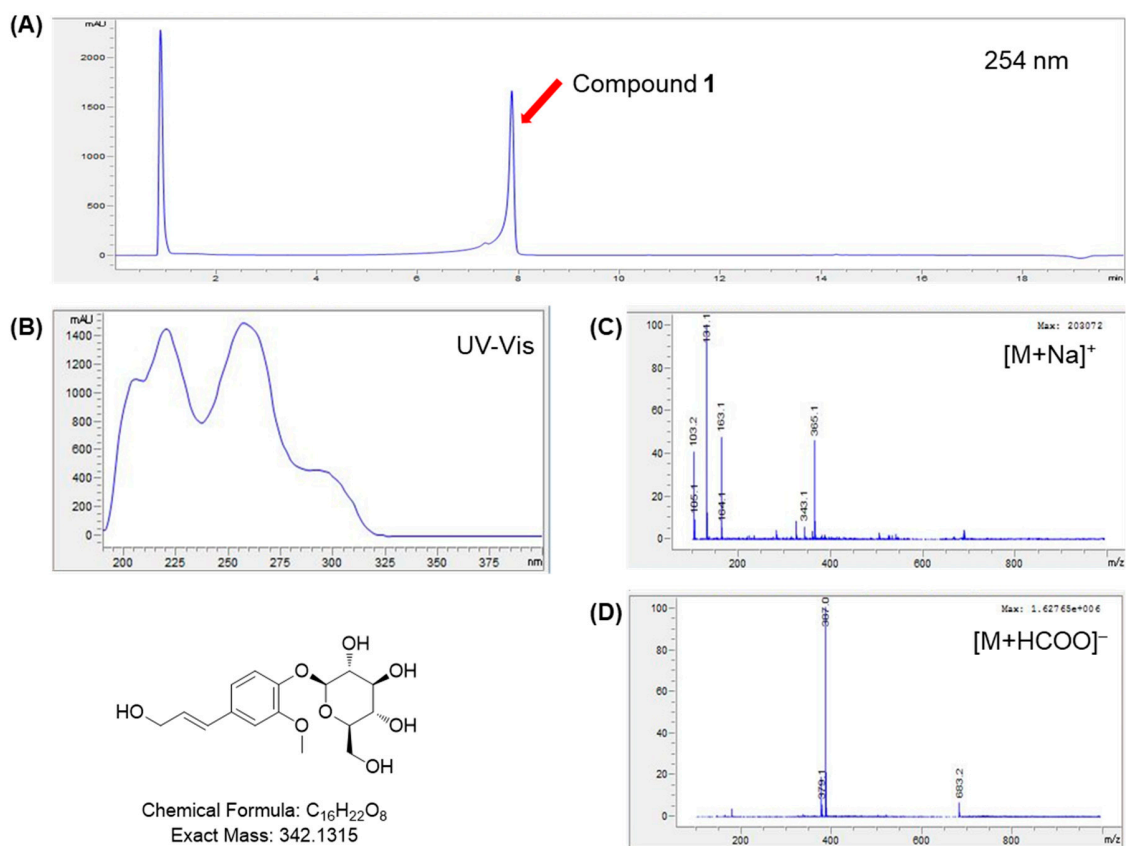


Figure S2. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for **1**.

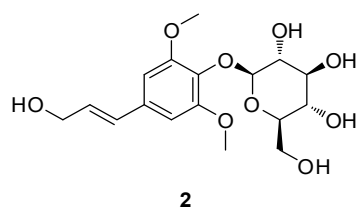
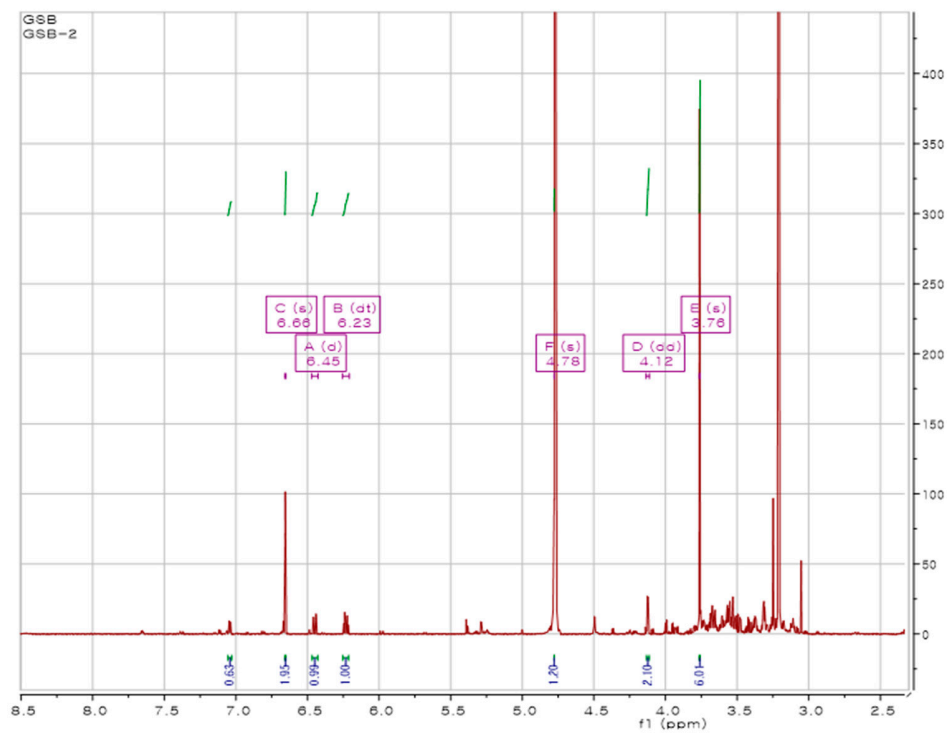


Figure S3. ^1H -NMR (CD_3OD , 850 MHz) spectrum of syringin (**2**).

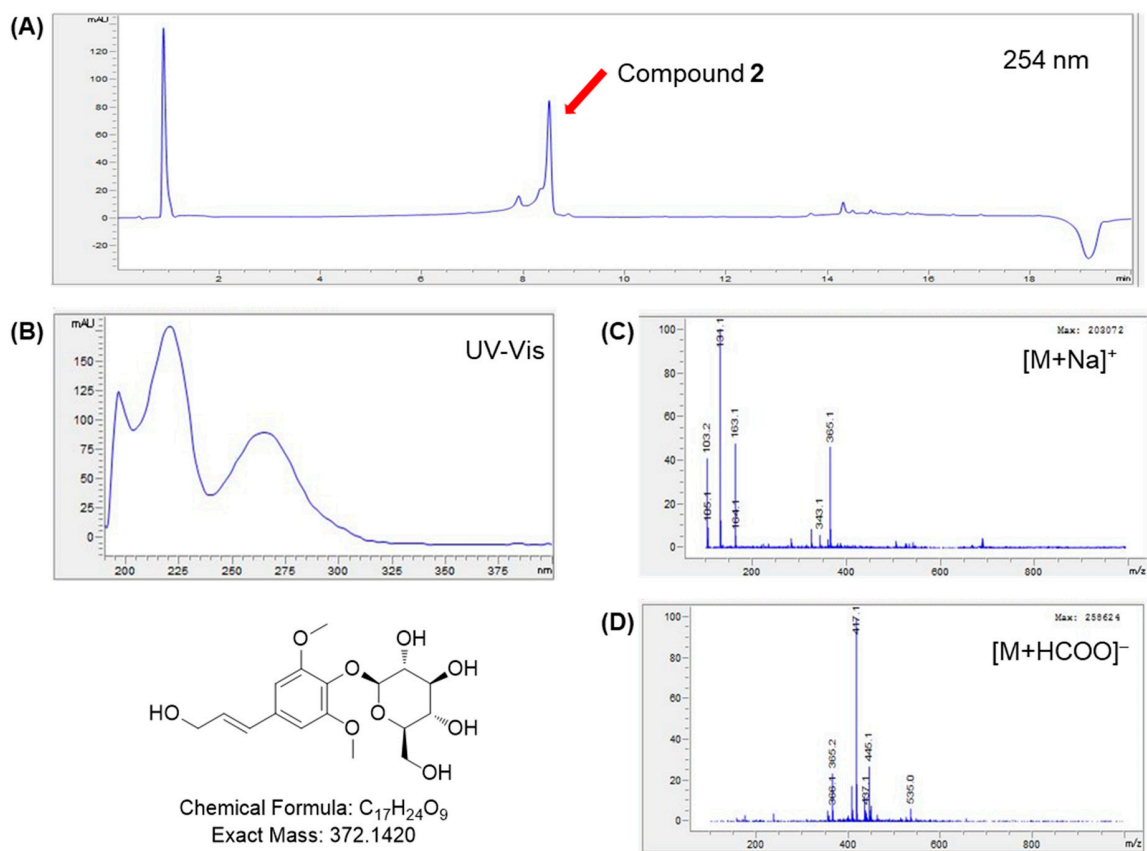


Figure S4. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 2.

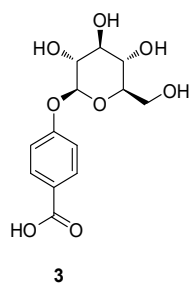
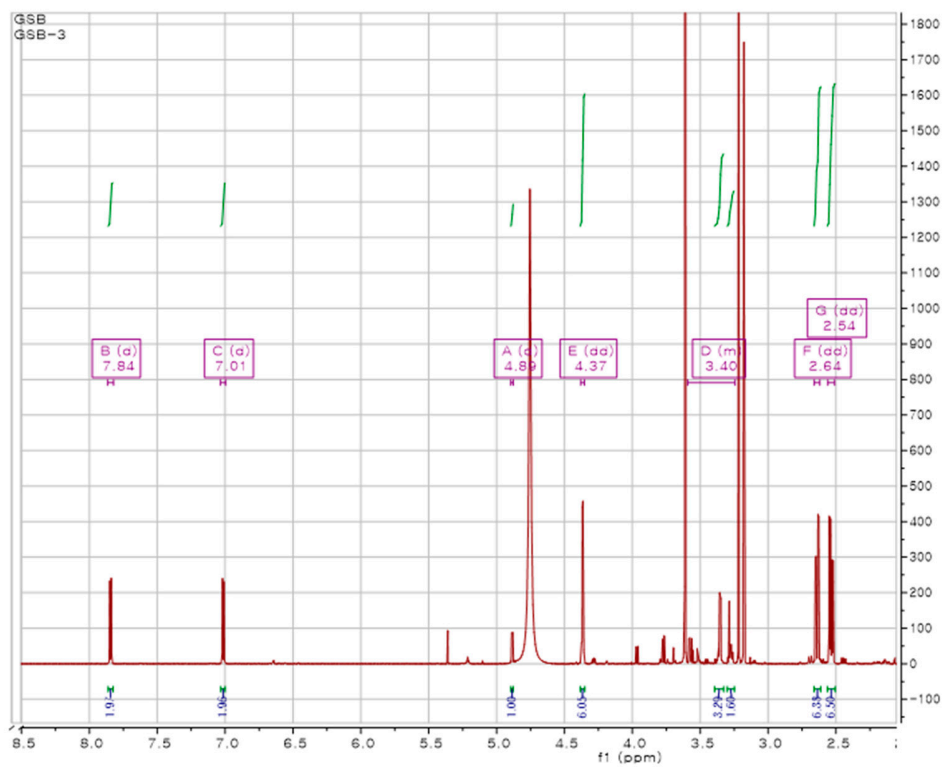


Figure S5 ^1H -NMR (CD_3OD , 850 MHz) spectrum of 4-hydroxybenzoic acid 4- O - β -D-glucopyranoside (**3**).

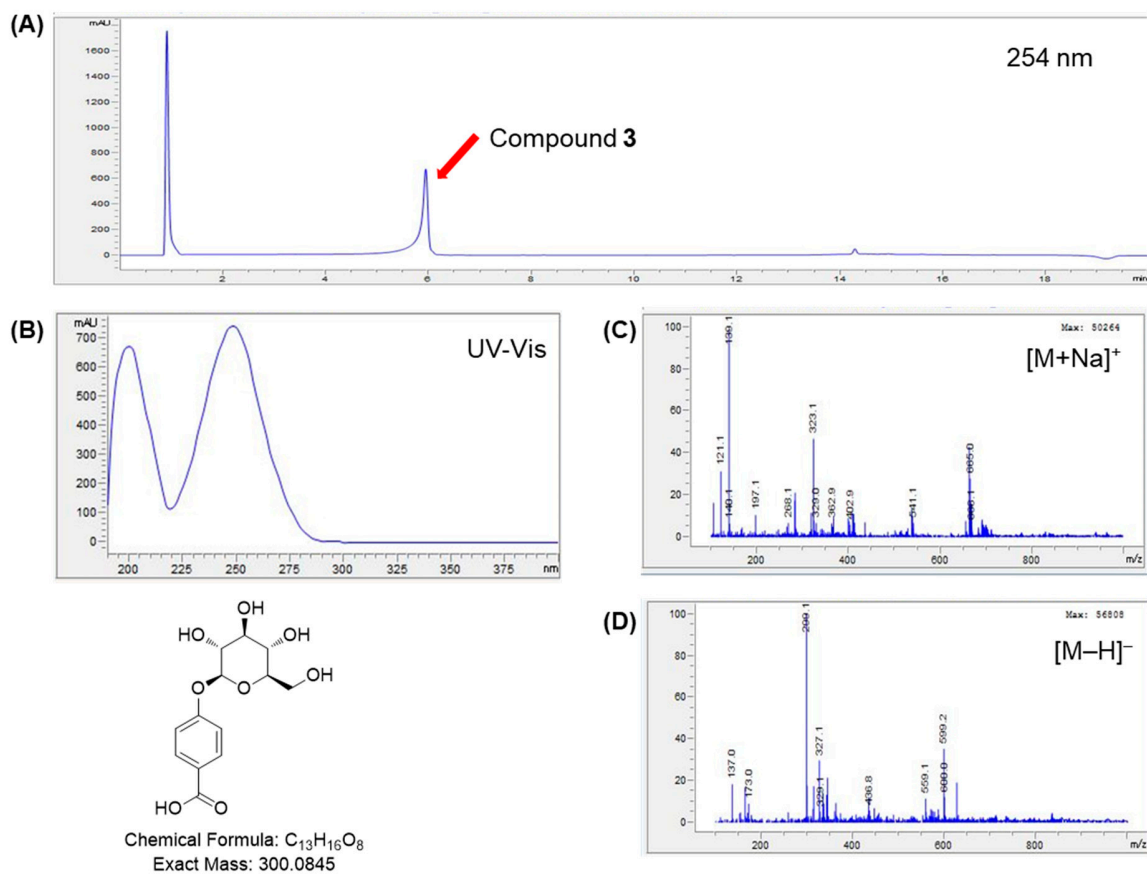


Figure S6. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for 3.

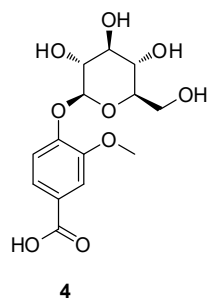
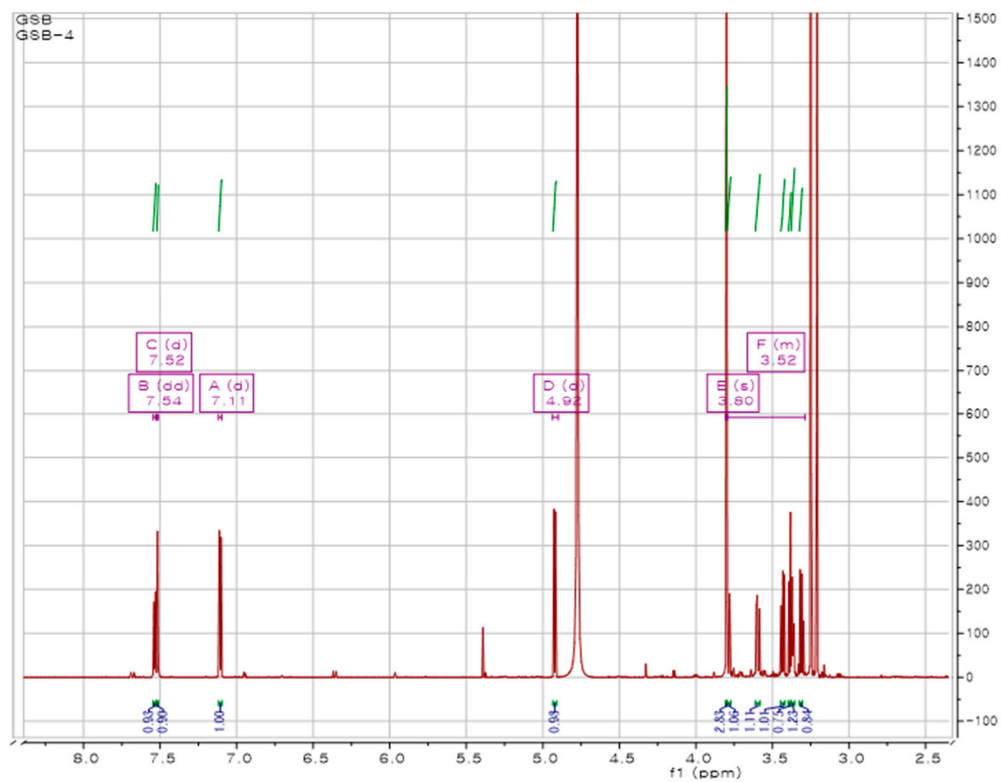


Figure S7. ^1H -NMR (CD_3OD , 850 MHz) spectrum of vanillic acid 4- O - β -D-glucopyranoside (**4**).

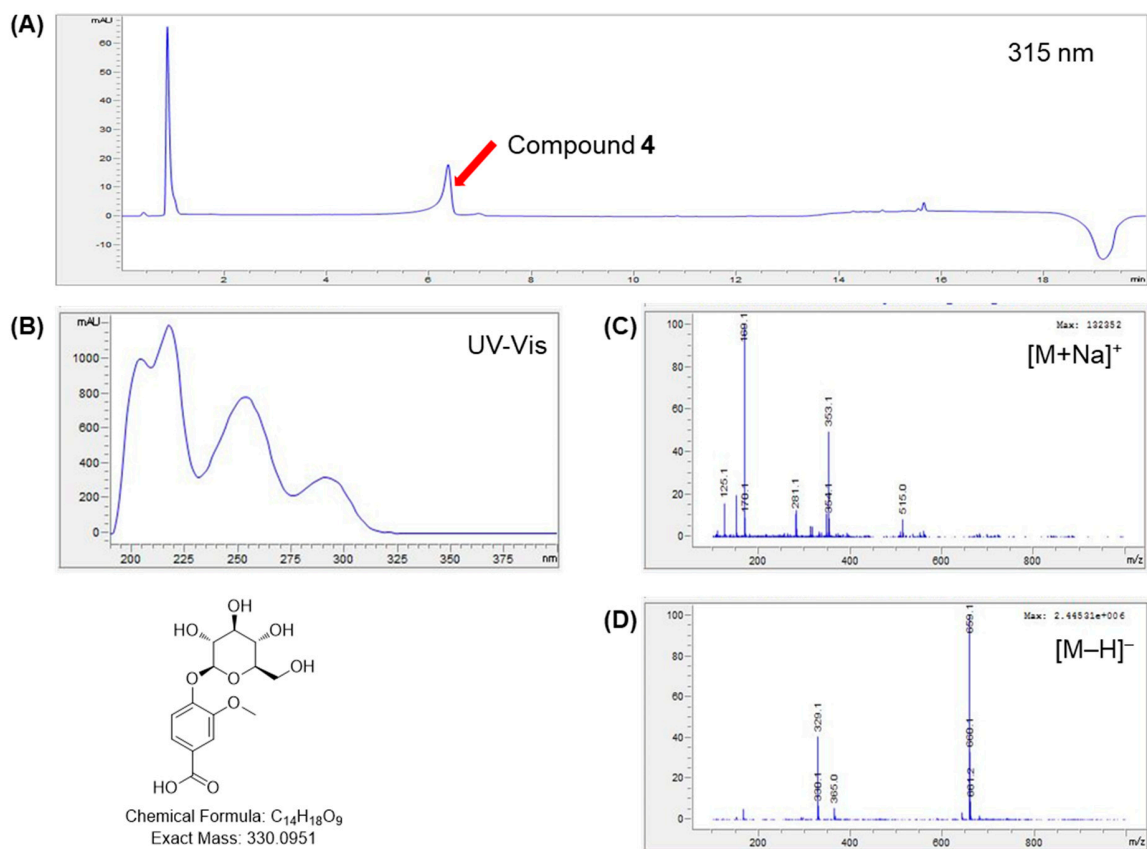


Figure S8. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for **4**.

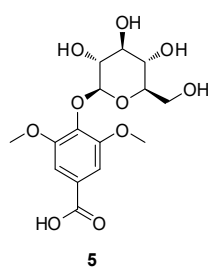
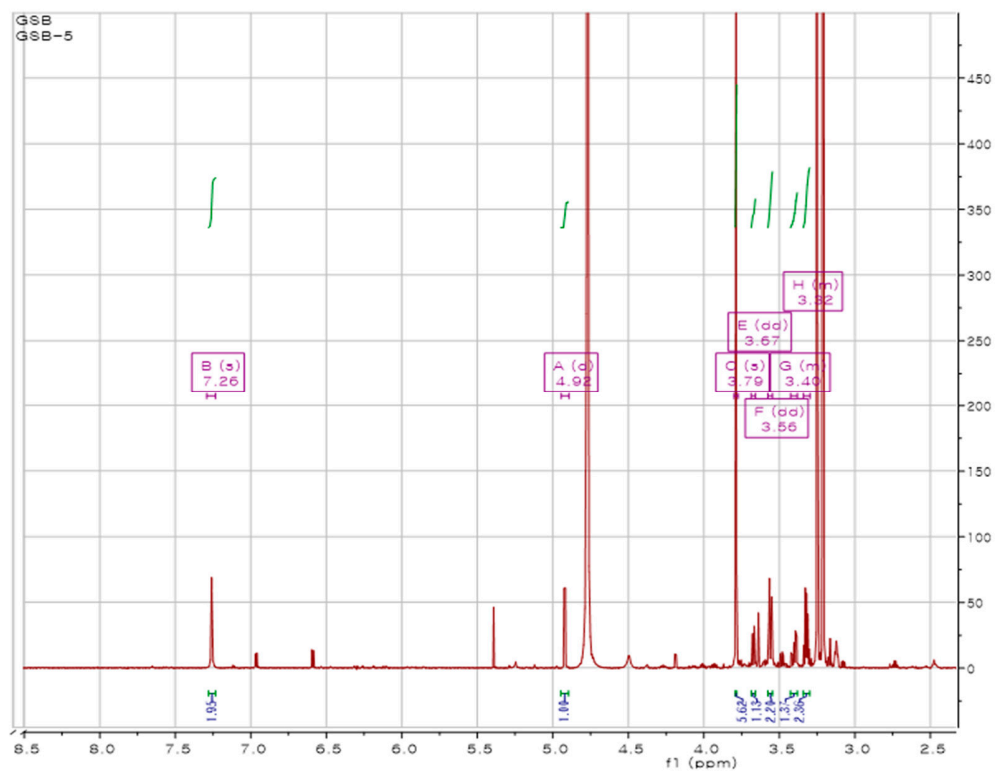


Figure S9. ^1H -NMR (CD_3OD , 850 MHz) spectrum of glucosyringic acid (**5**).

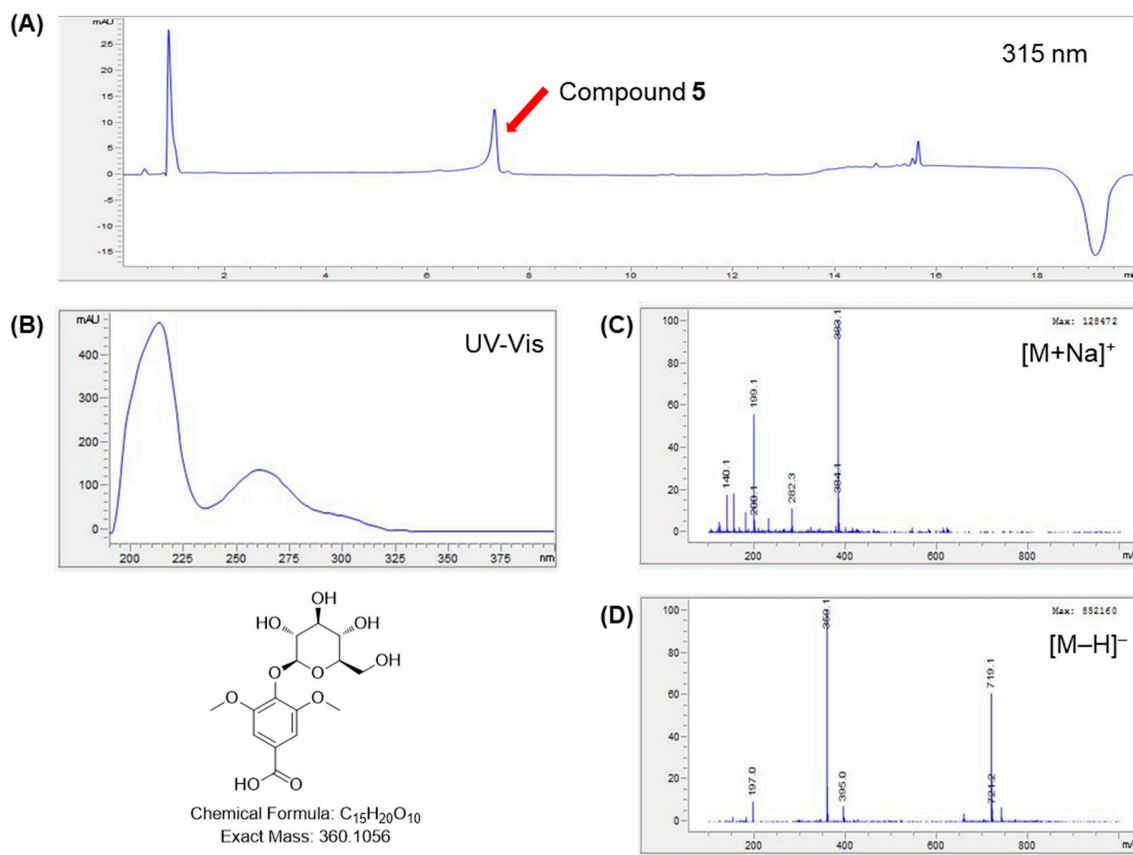


Figure S10. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for **5**.

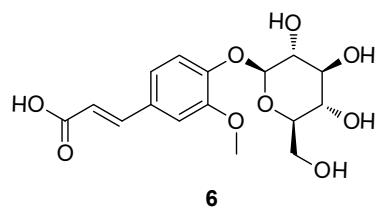


Figure S11. ^1H -NMR (CD_3OD , 850 MHz) spectrum of (*E*)-ferulic acid 4-*O*- β -D-glucoside (**6**).

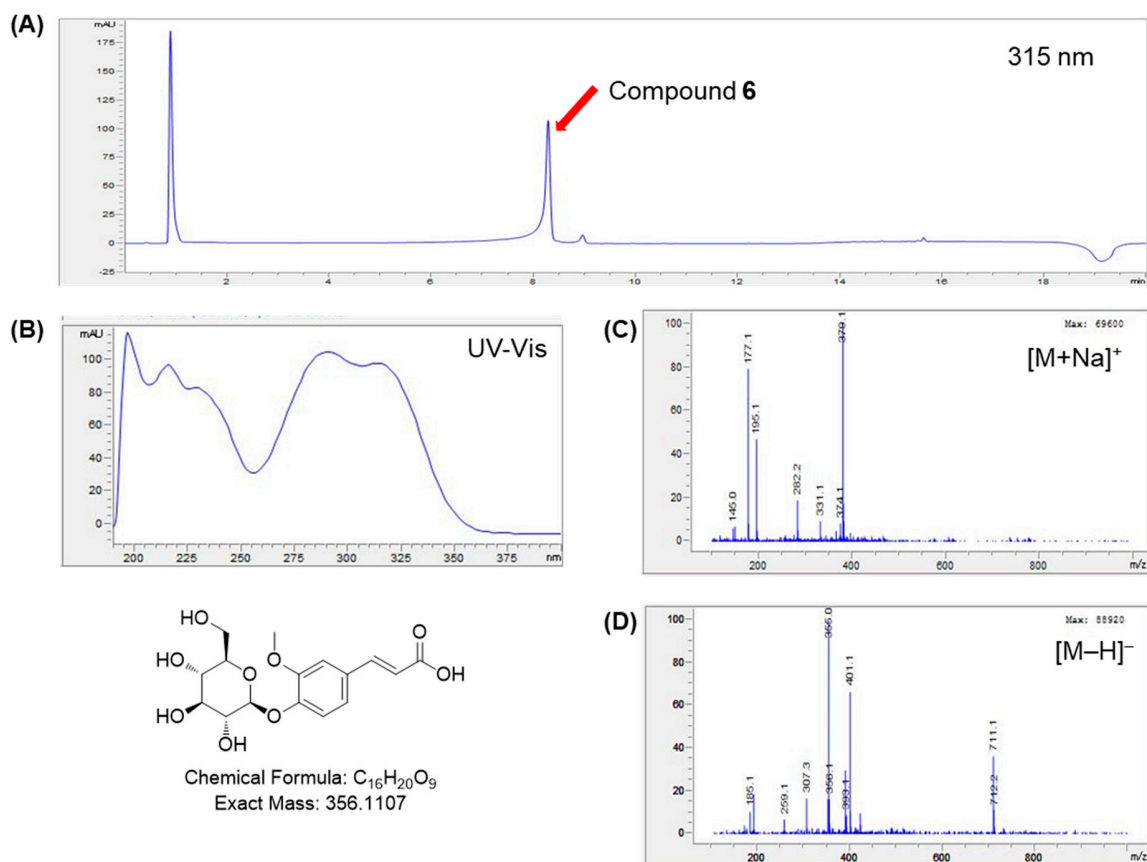


Figure S12. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for **6**.

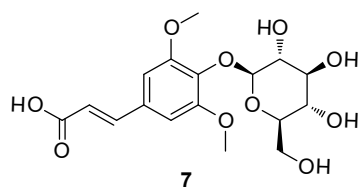
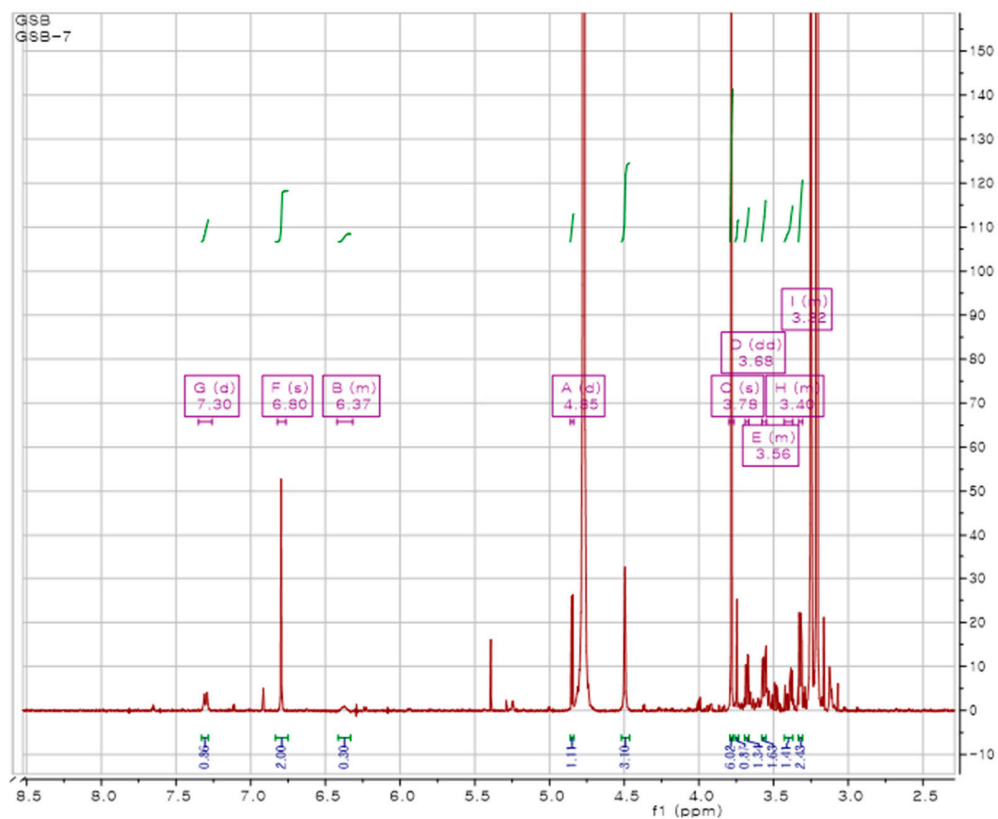


Figure S13. ^1H -NMR (CD_3OD , 850 MHz) spectrum of (*E*)-sinapic acid 4- O - β -D-glucopyranoside (**7**).

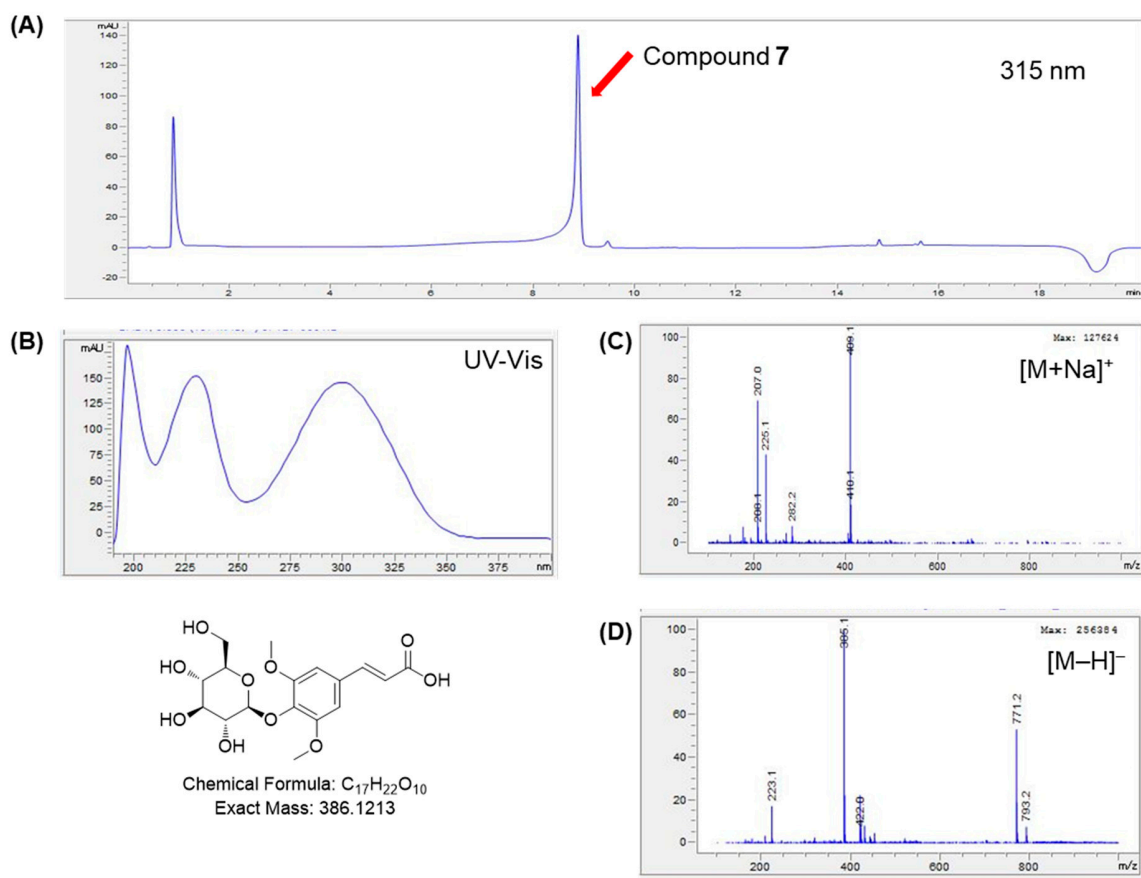


Figure S14. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for 7.

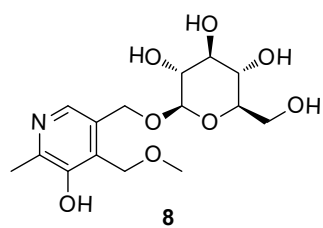
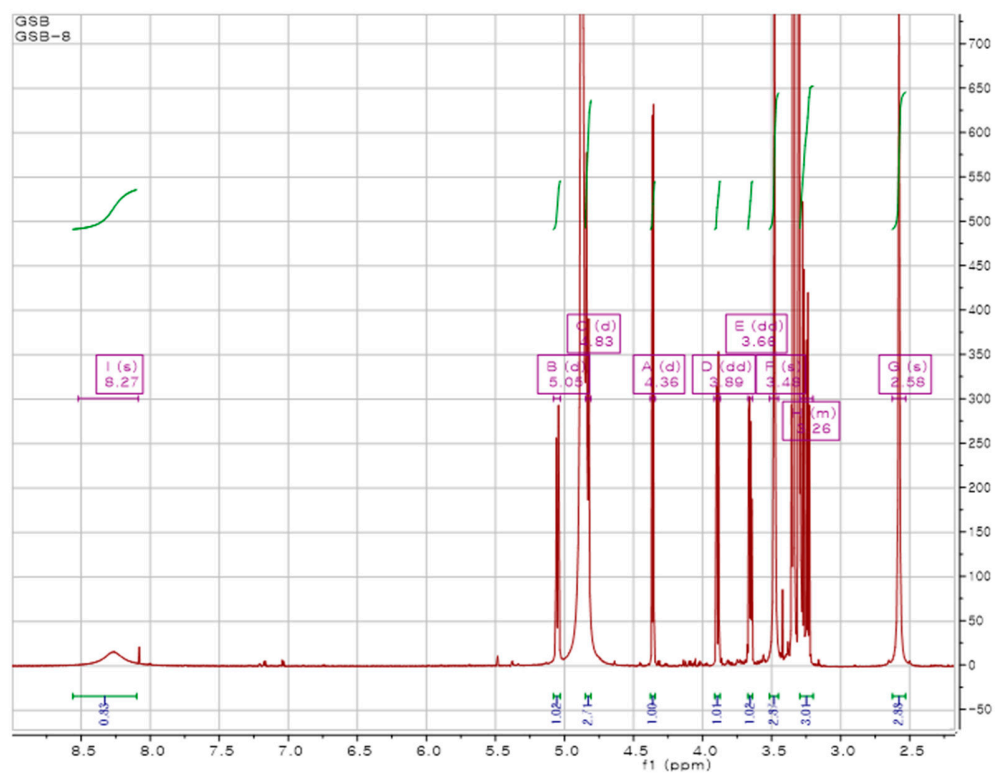


Figure S15. ^1H -NMR (CD_3OD , 850 MHz) spectrum of ginkgotoxin-5-glucoside (**8**).

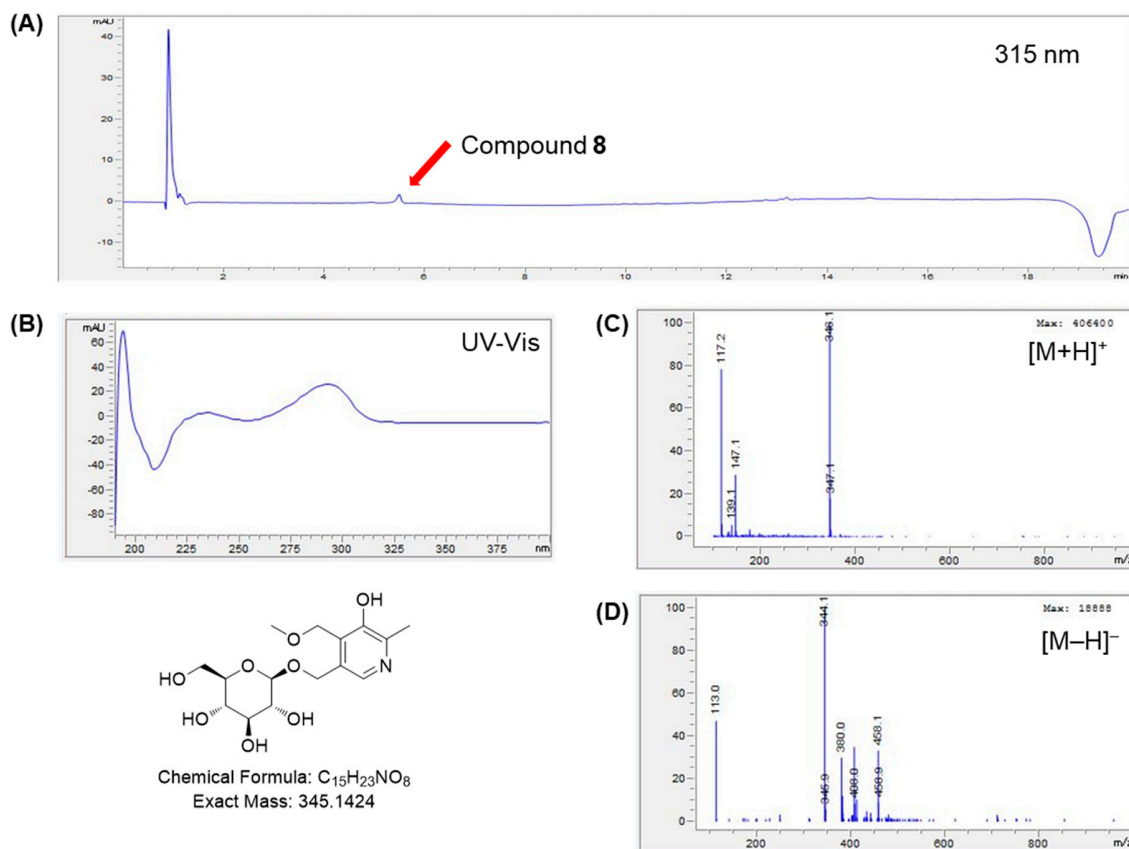


Figure S16. UV chromatogram of LC/MS (A: monitored at 315 nm) and UV (B) and MS data (C: positive; D: negative) for 8.

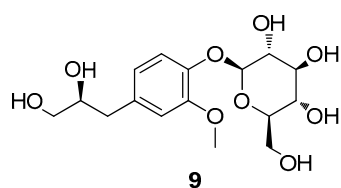
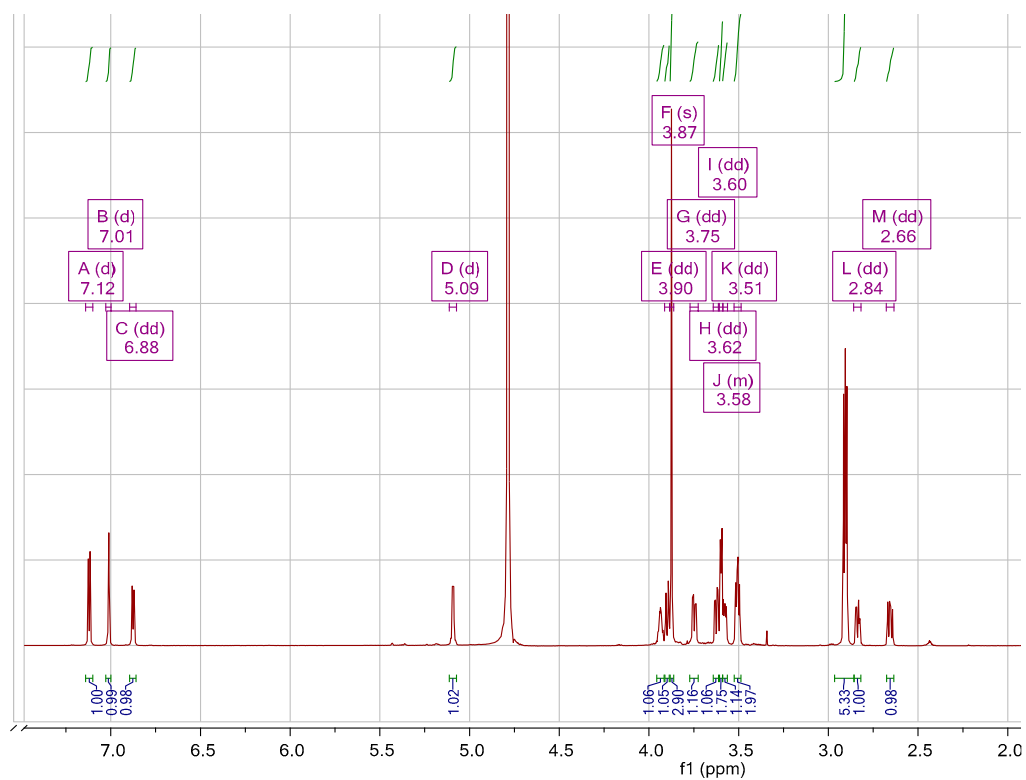


Figure S17. ^1H -NMR (CD_3OD , 850 MHz) spectrum of ginkgopanoside (**9**).

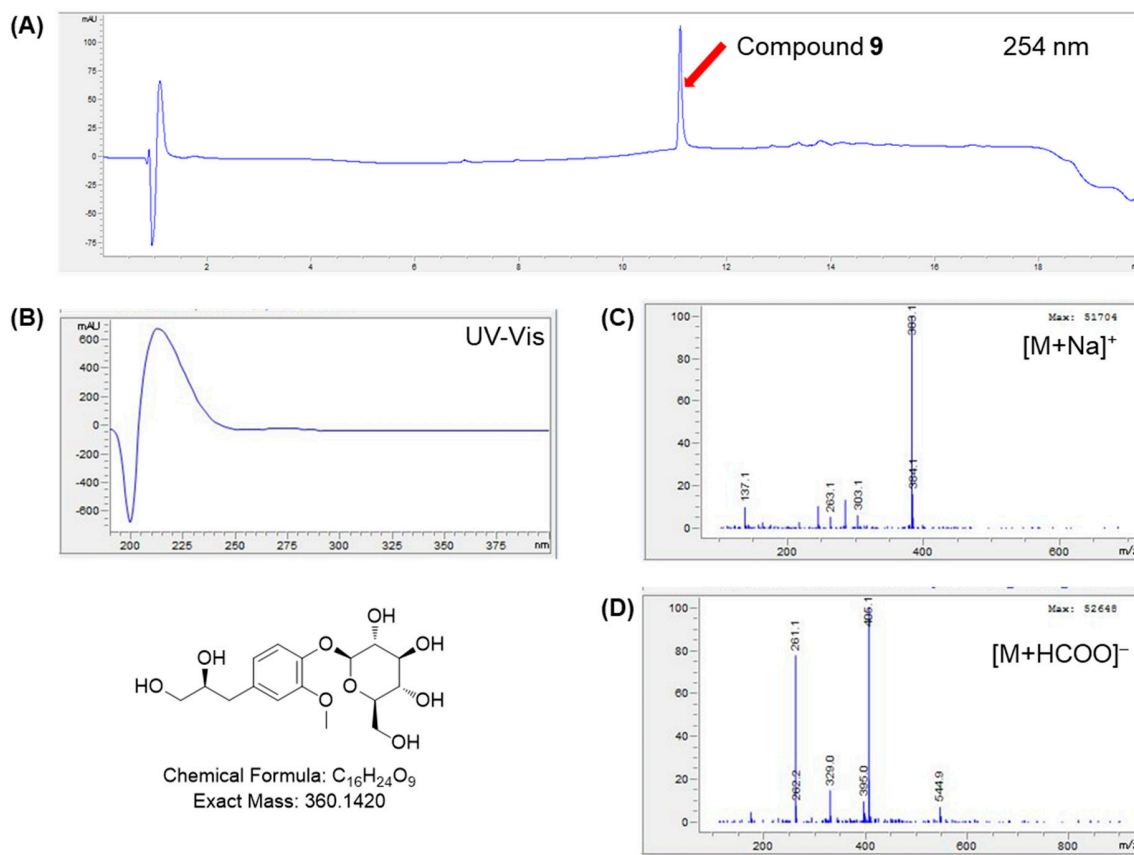


Figure S18. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for **9**.

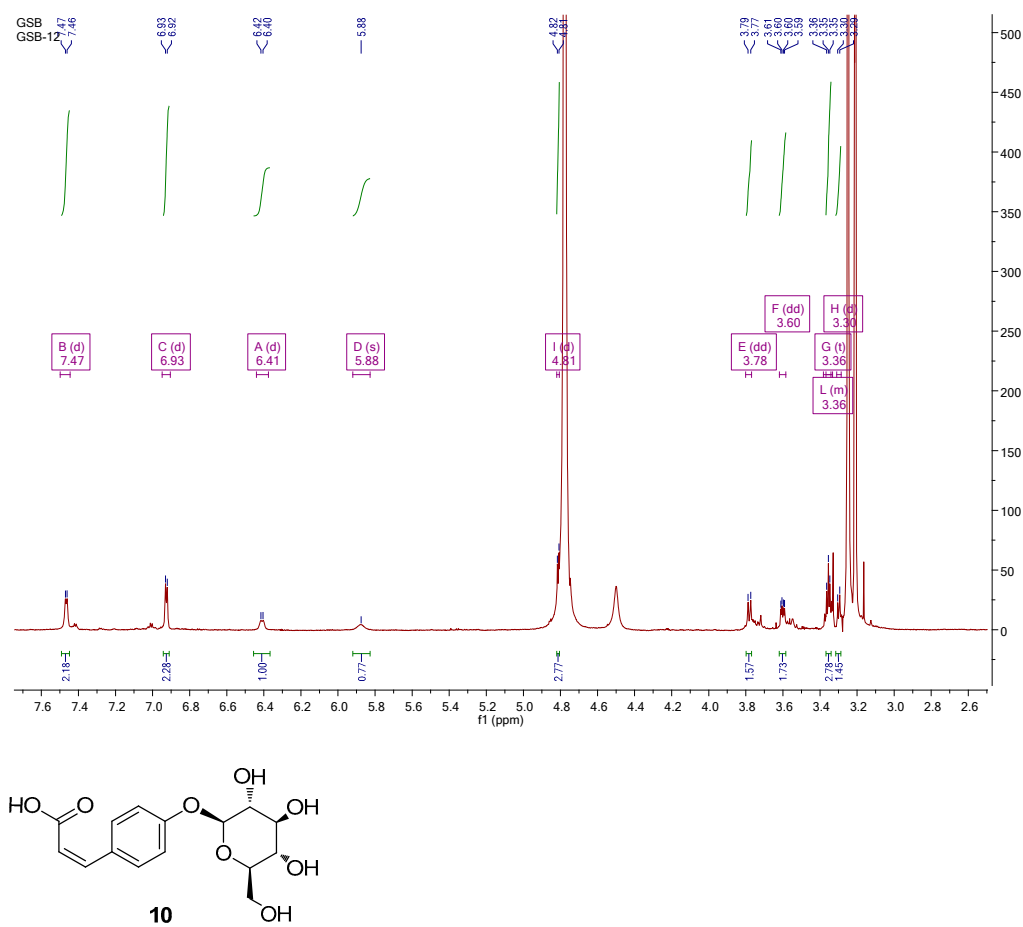


Figure S19. ¹H-NMR (CD₃OD, 850 MHz) spectrum of (Z)-4-coumaric acid 4-O-β-D-glucopyranoside (**10**).

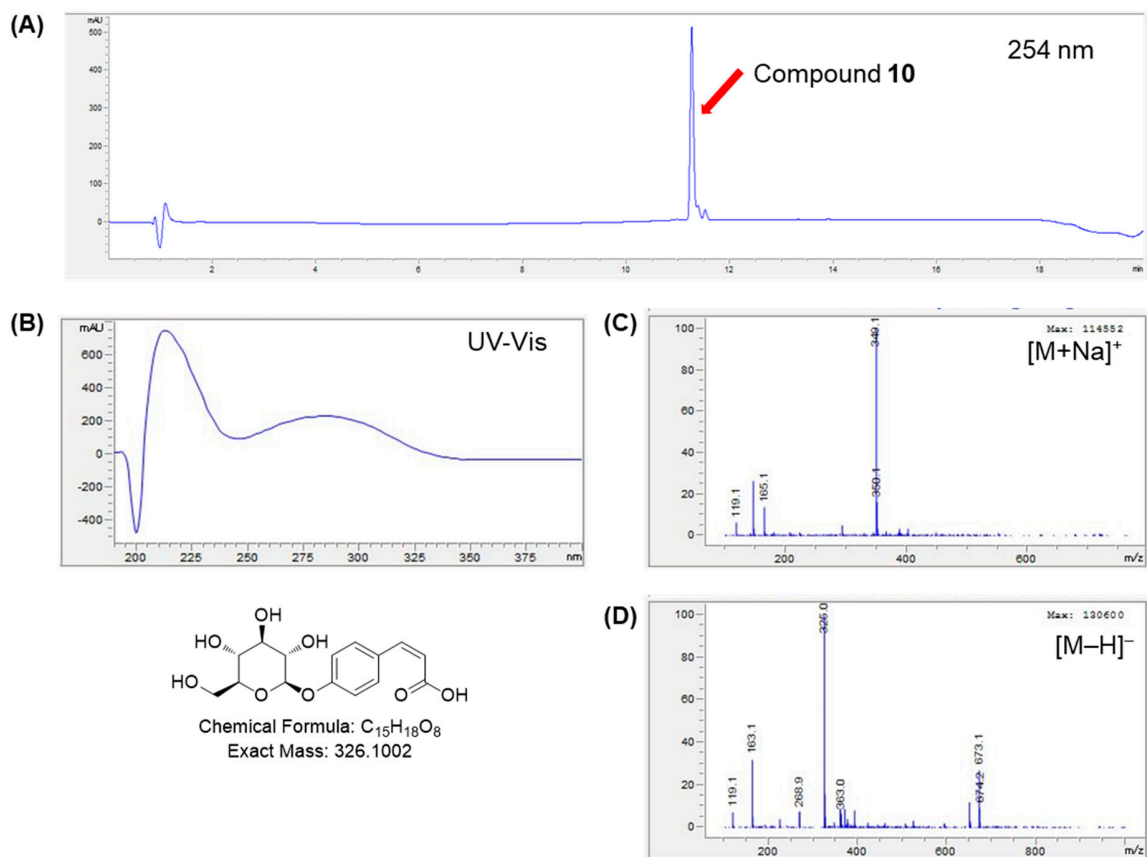


Figure S20. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for **10**.

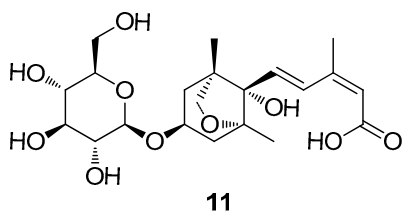
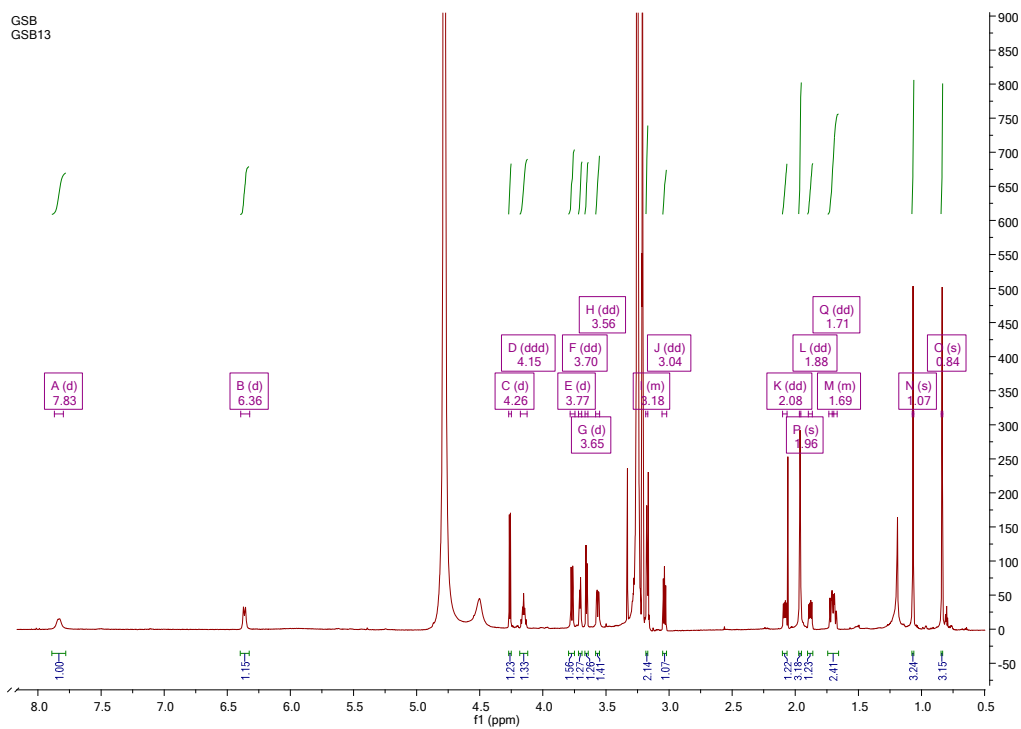


Figure S21. ^1H -NMR (CD_3OD , 850 MHz) spectrum of (1'*R*,2'*S*,5'*R*,8'*S*,2'*Z*,4'*E*)-dihydrophaseic acid 3'-*O*- β -D-glucopyranoside (**11**).

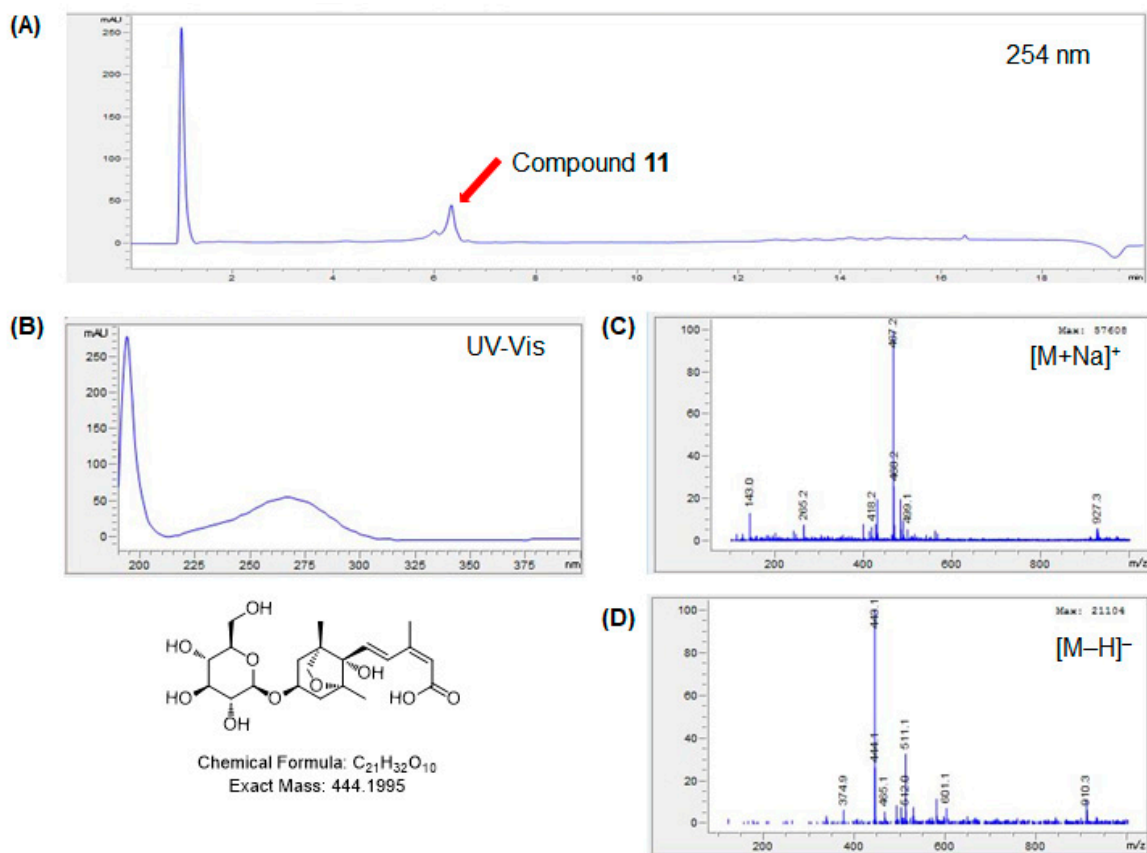


Figure S22. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for **11**.

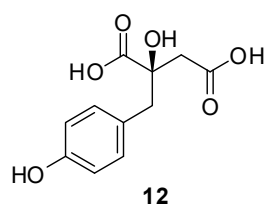
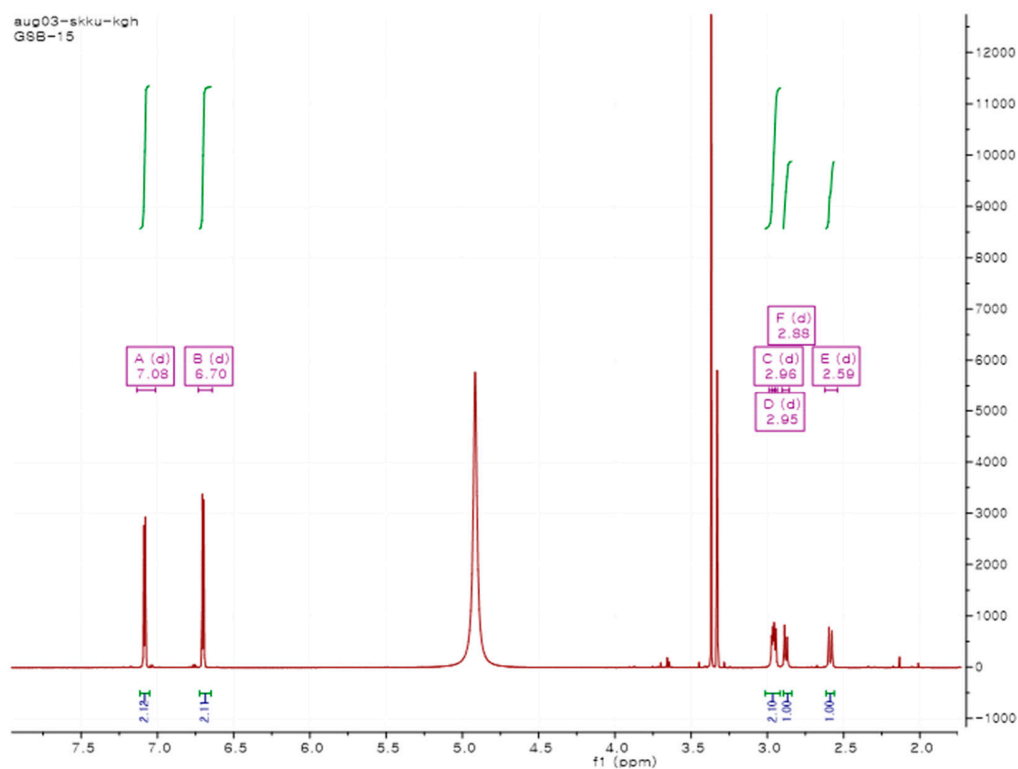


Figure S23. ^1H -NMR (CD_3OD , 850 MHz) spectrum of eucomic acid (**12**).

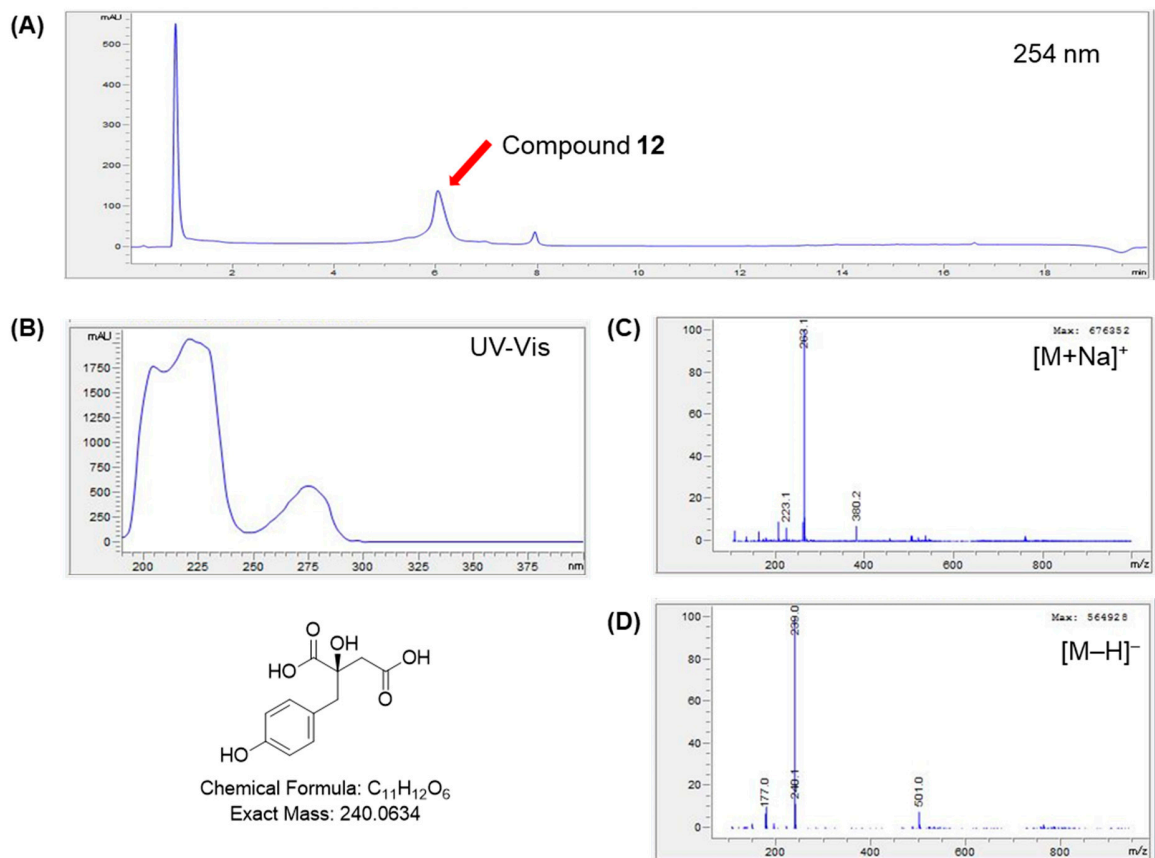


Figure S24. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for **12**.

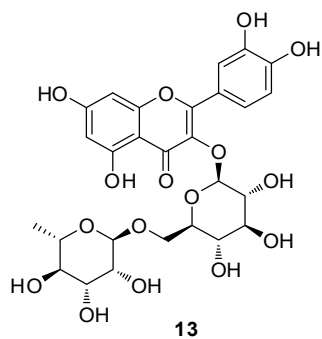
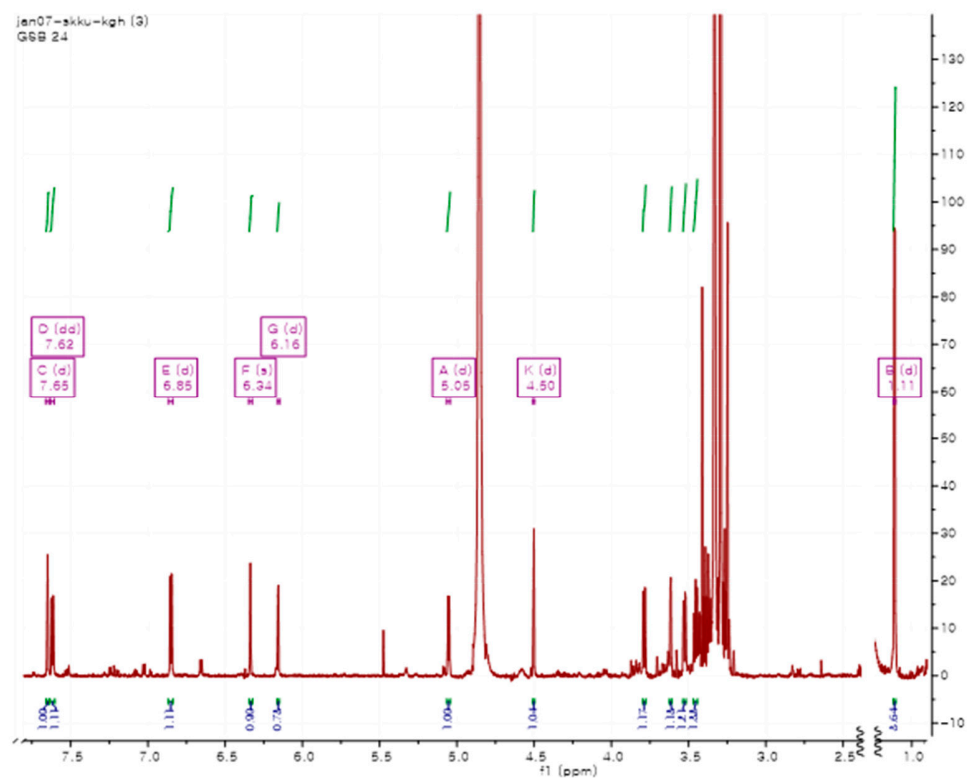


Figure S25. ^1H -NMR (CD_3OD , 850 MHz) spectrum of rutin (**13**).

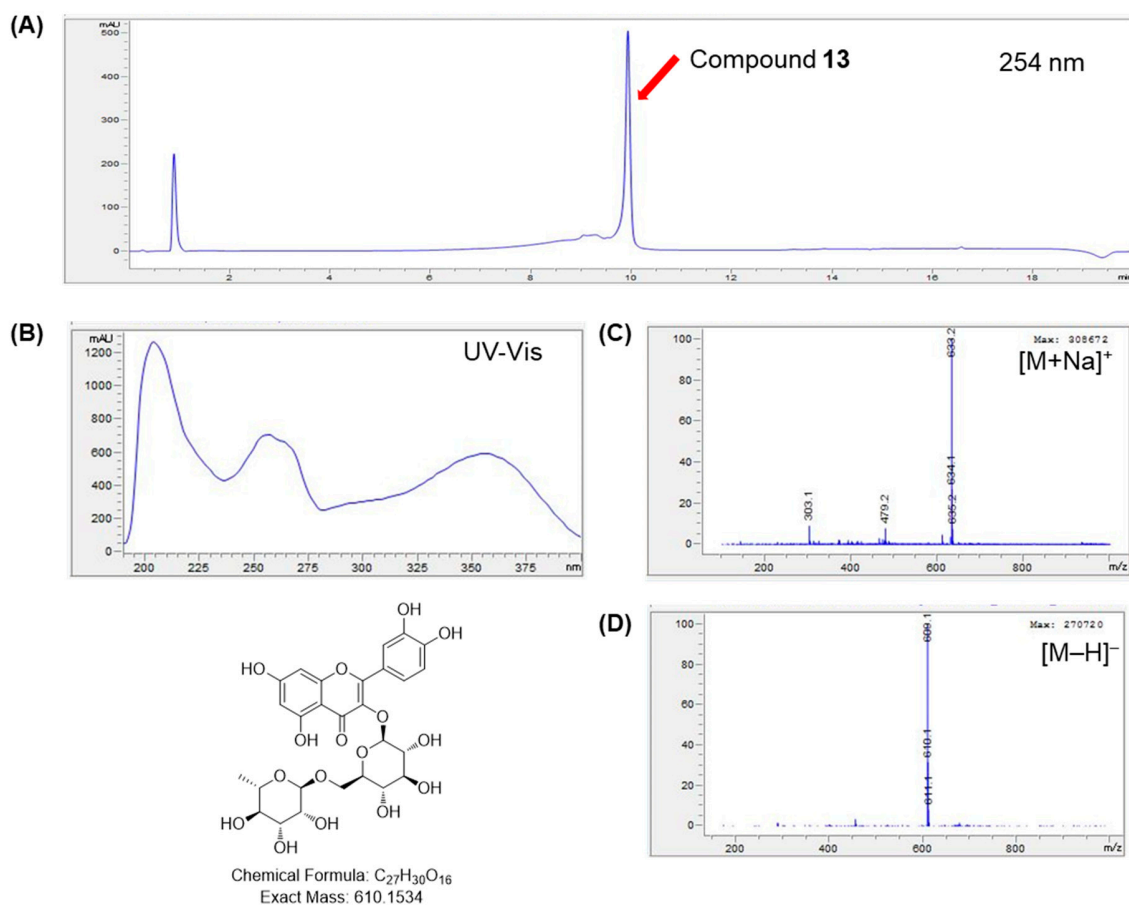


Figure S26. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for **13**.

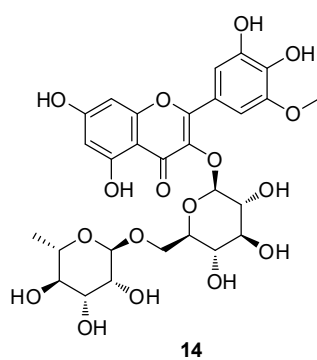
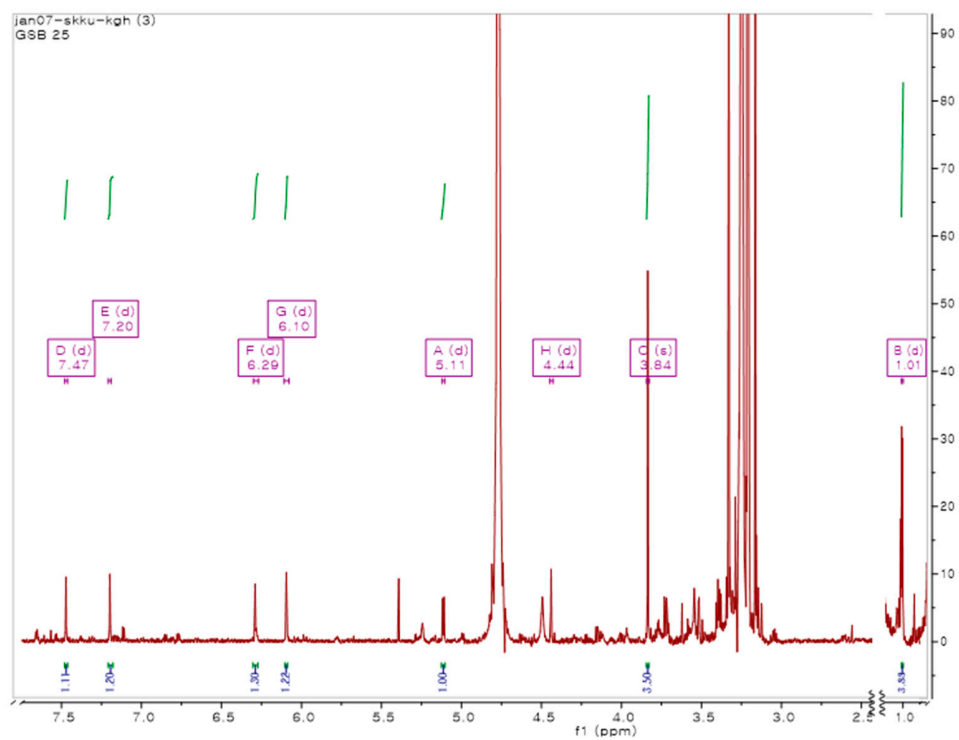


Figure S27. ^1H -NMR (CD_3OD , 850 MHz) spectrum of laricitrin 3-rutinoside (**14**).

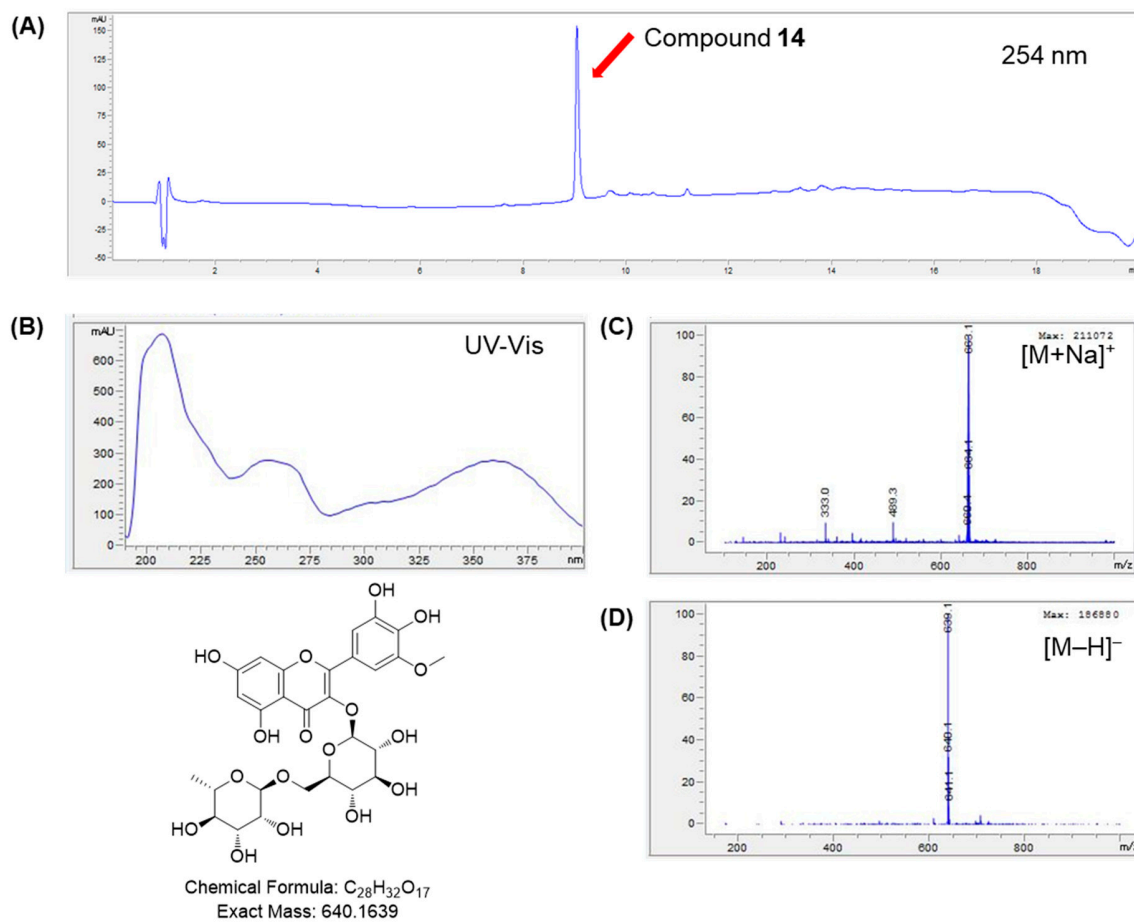


Figure S28. UV chromatogram of LC/MS (A: monitored at 254 nm) and UV (B) and MS data (C: positive; D: negative) for **14**.

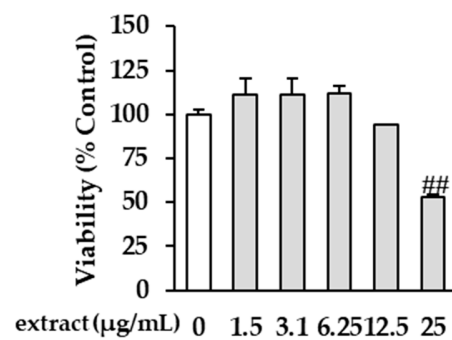


Figure S29. Effect of the extract on NHDF viability. Data are presented as mean \pm SEM ($n = 2$). ^{##} $p < 0.01$ compared with the control group.

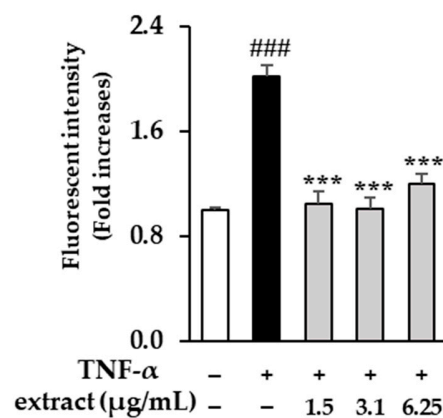


Figure S30. Effects of the extract on TNF- α -induced intercellular reactive oxygen species (ROS) generation. Data are presented as the mean \pm SEM (n = 3). ### p < 0.001 compared to the control group; *** p < 0.001 compared to the TNF- α -treated group.

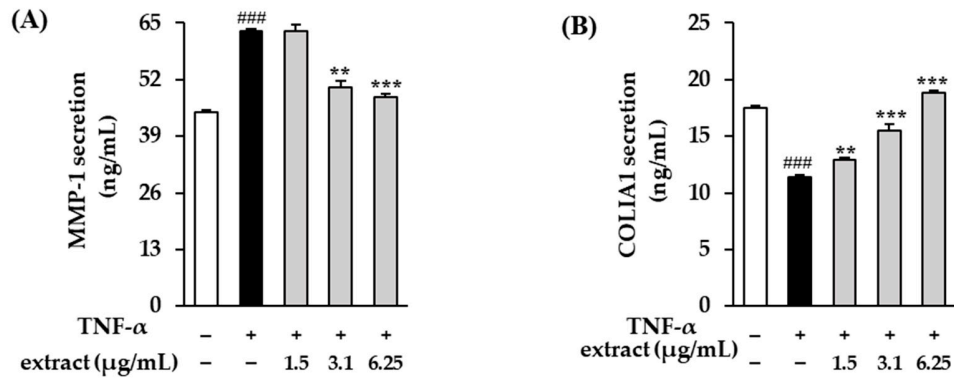


Figure S31. Effect of the extract on MMP-1 and COLIA1 protein expression in tumor necrosis factor- α (TNF- α)-stimulated normal human dermal fibroblasts (NHDFs). Data are presented as the mean \pm SEM (n = 3). ### p < 0.001 compared to the control group; ** p < 0.01, and *** p < 0.001, compared to the TNF- α -treated group.