

Effect of light, methyl jasmonate and cyclodextrin on production of phenolic compounds in hairy root cultures of *Scutellaria lateriflora*

Zachary Marsh^{a,b}, Tianhong Yang^{a,b}, Luis Nopo-Olazabal^{a,b}, Shuchi Wu^a, Taylor Ingle^{a,1},
Nirmal Joshee^c and Fabricio Medina-Bolivar^{a,b,*}

^aArkansas Biosciences Institute, Arkansas State University, State University, AR 72467, USA

^bDepartment of Biological Sciences, Arkansas State University, State University, AR 72467, USA

^cAgricultural Research Station, Fort Valley State University, Fort Valley, GA 31030, USA

*Corresponding author:

Fabricio Medina-Bolivar, Ph.D.

Arkansas Biosciences Institute, Arkansas State University, P.O. Box 639, State University, AR 72467, United States.

Tel.: +1 870 680 4319; fax +1 870 680 4348.

E-mail address: fmedinabolivar@astate.edu

¹Current address: National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA

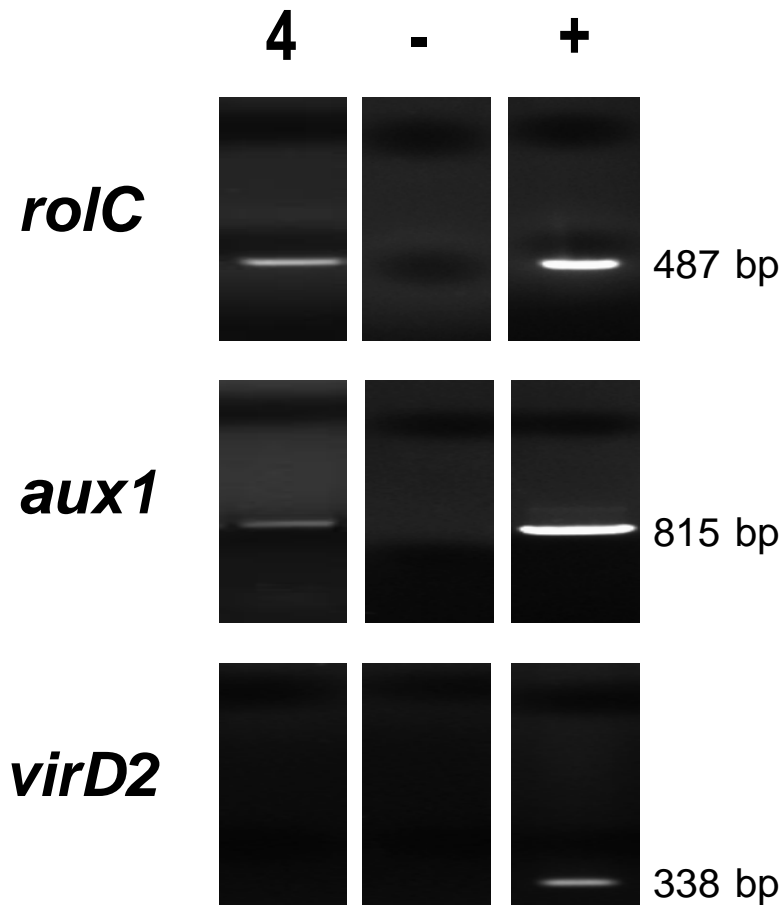


Figure S1. Molecular characterization of *Scutellaria lateriflora* hairy root line 4. Genomic DNA extracted from the root tissue was analyzed by PCR. Analysis shows the presence of the *rolC* and *aux1* genes from the T-DNA of *A. rhizogenes*. A negative PCR result for the *virD2* gene confirms that no *Agrobacterium* remained in the root tissue. Each reaction includes + (Ri plasmid) and - (no template) control.

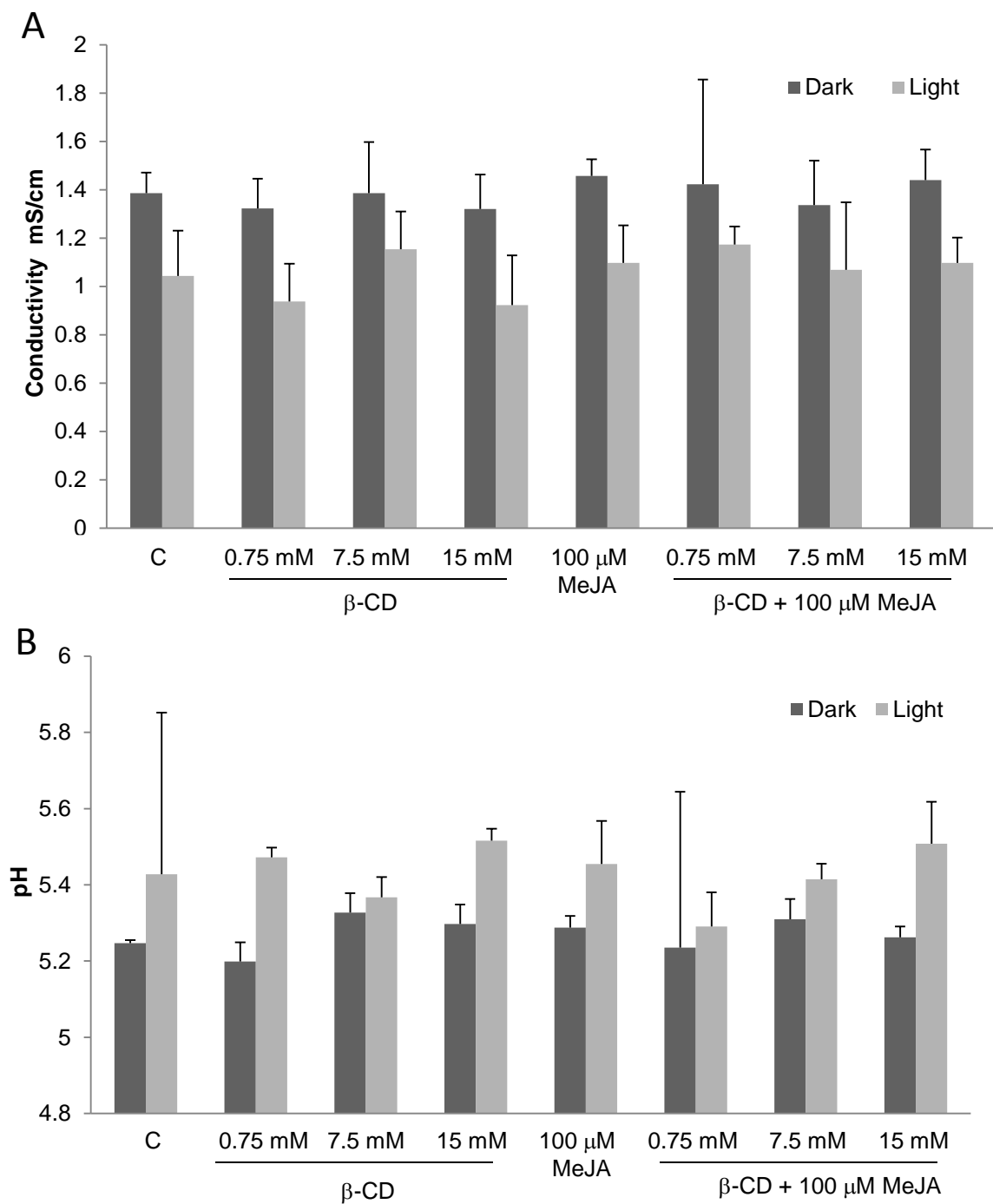


Figure S2. Values of conductivity (**A**) and pH (**B**) of the spent medium of hairy root cultures of *Scutellaria lateriflora* line 4 before elicitor treatment. Cultures were incubated for 30 days under continuous light or darkness. Each value represents the average + standard deviation of three biological replicates. C, control; β-CD, methyl-β-cyclodextrin; MeJA, methyl jasmonate.

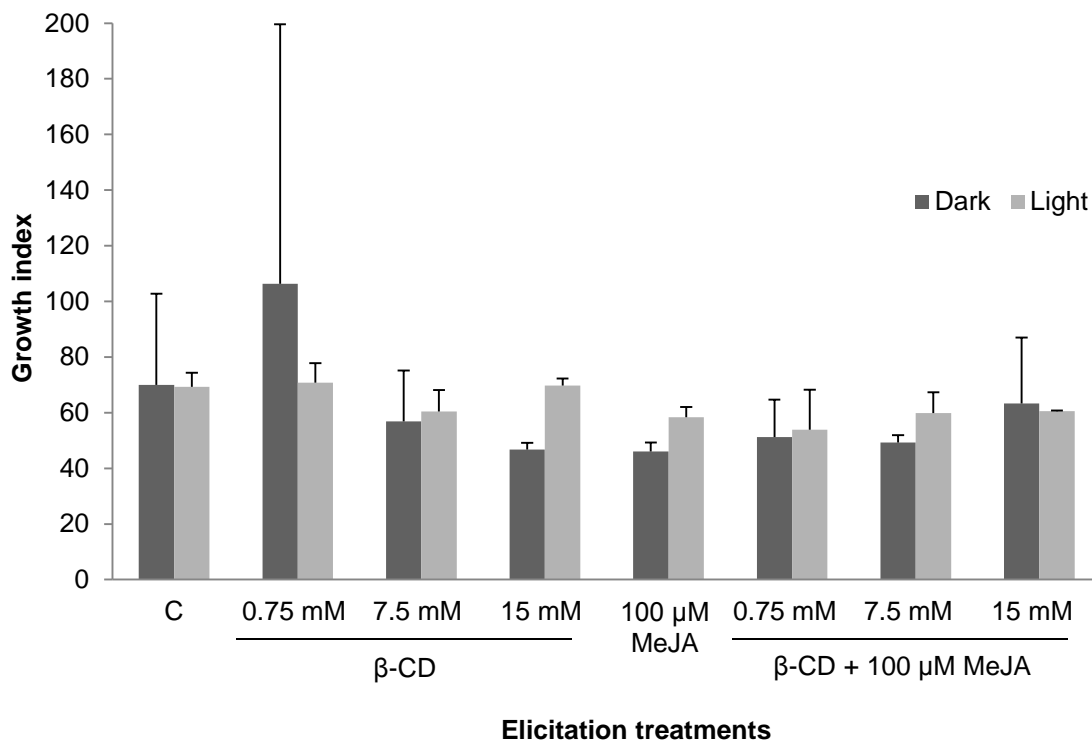
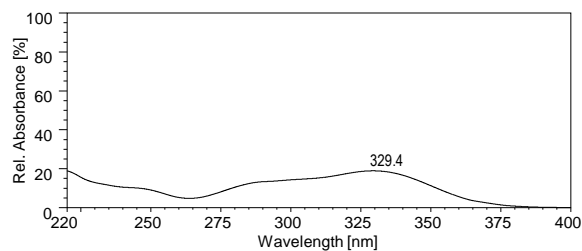
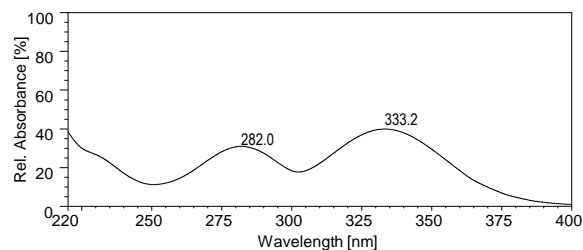


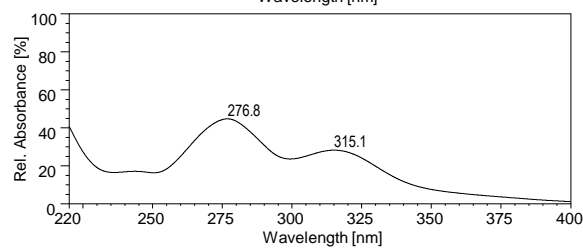
Figure S3. Growth index of *Scutellaria lateriflora* hairy roots line 4 grown under either continuous darkness or light. Each treatment represents the average + standard deviation of three biological replicates. Cultures were elicited with methyl- β -cyclodextrin (β -CD) alone or in combination with methyl jasmonate (MeJA) for 24 h before harvesting of root tissue.



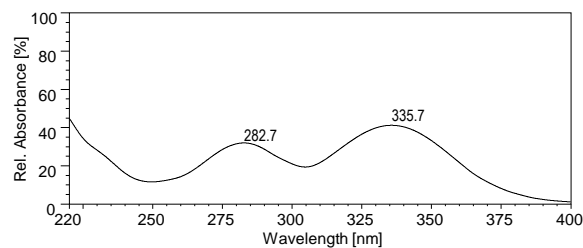
Verbascoside (1): Rt 4.55 min



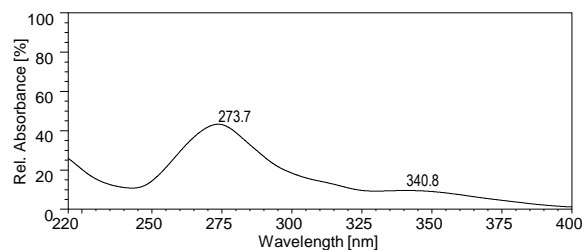
Scutellarin (2): Rt 5.14 min



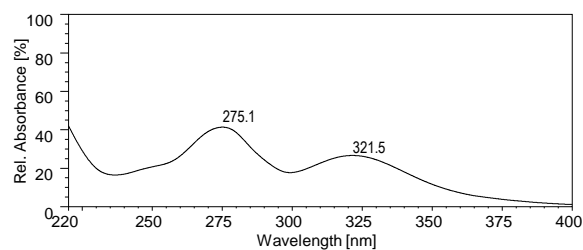
Baicalin (3): Rt 7.62 min



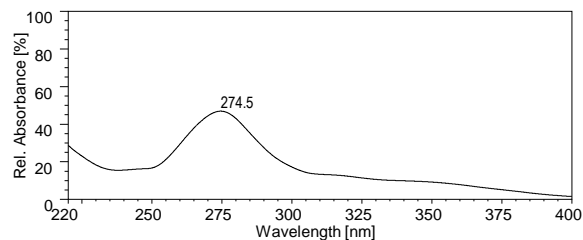
Scutellarein (4): Rt 7.87 min



Wogonoside (5): Rt 9.67 min



Baicalein (6): Rt 12.28 min



Wogonin (7): Rt 15.60 min

Figure S4: UV spectra and retention times of the seven phenolics identified in the hairy root tissue of *S. lateriflora* line 4. Rt: HPLC retention time.

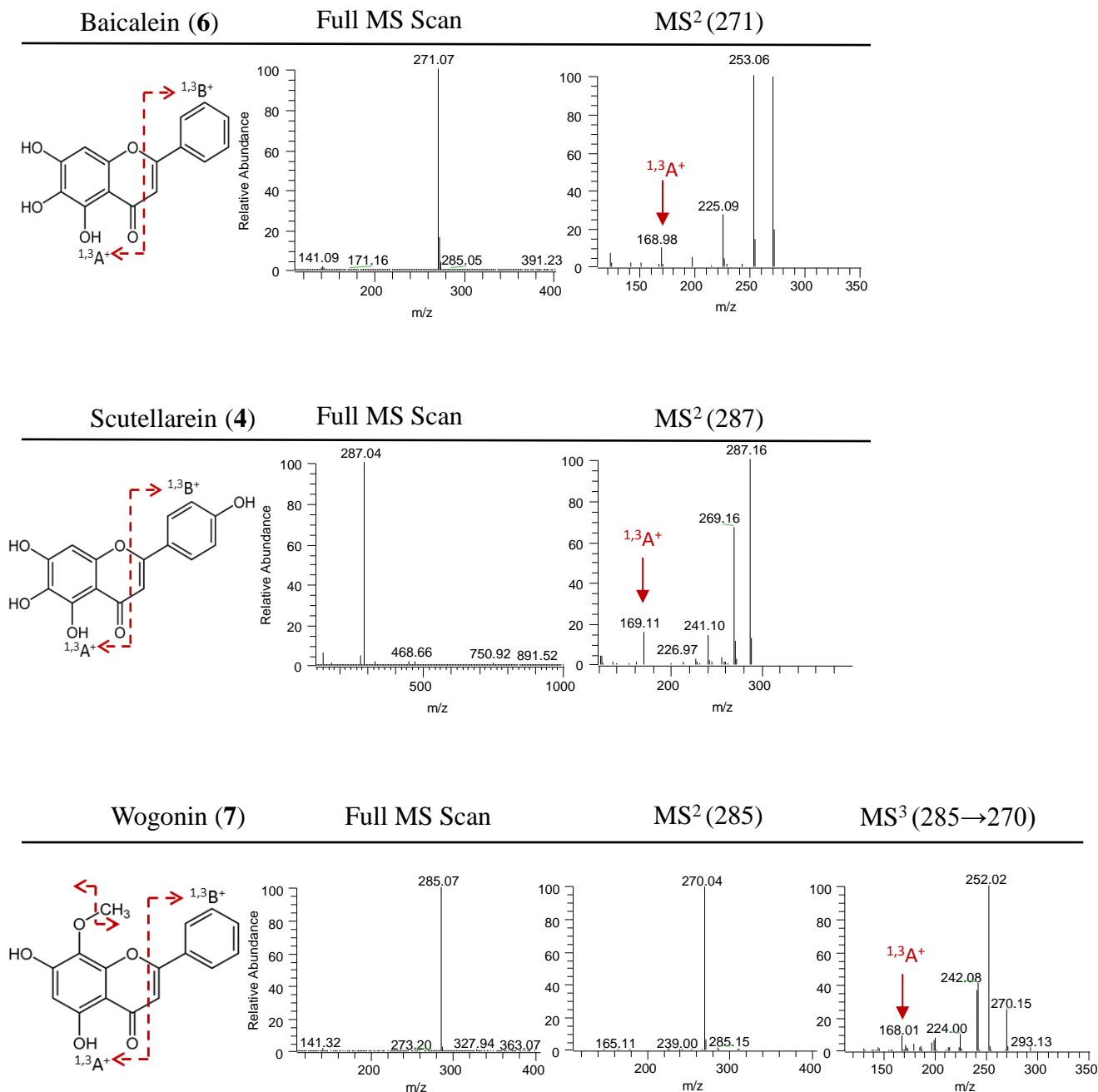


Figure S5: LC-mass spectrometry analyses of baicalein (6), scutellarein (4) and wogonin (7). *Left:* Chemical structure and fragmentation pattern. *Right:* Full MS scan and MSⁿ spectra of each standard under positive ion mode with an ESI source.

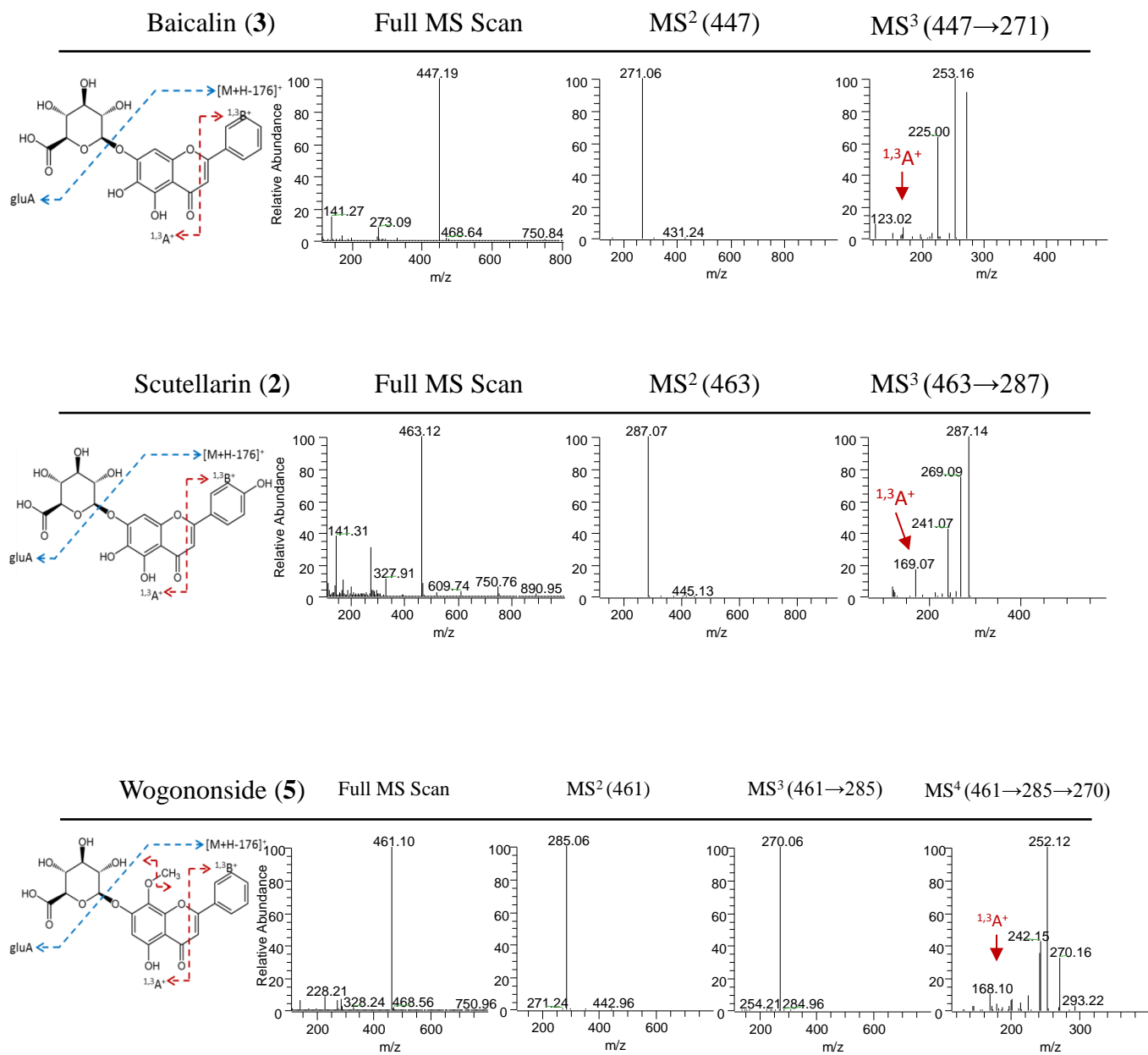


Figure S6: LC-mass spectrometry analyses of baicalin (3), scutellarin (2) and wogonoside (5). *Left:* Chemical structure and fragmentation pattern. *Right:* Full MS scan and MSⁿ spectra of each standard under positive ion mode with an ESI source.

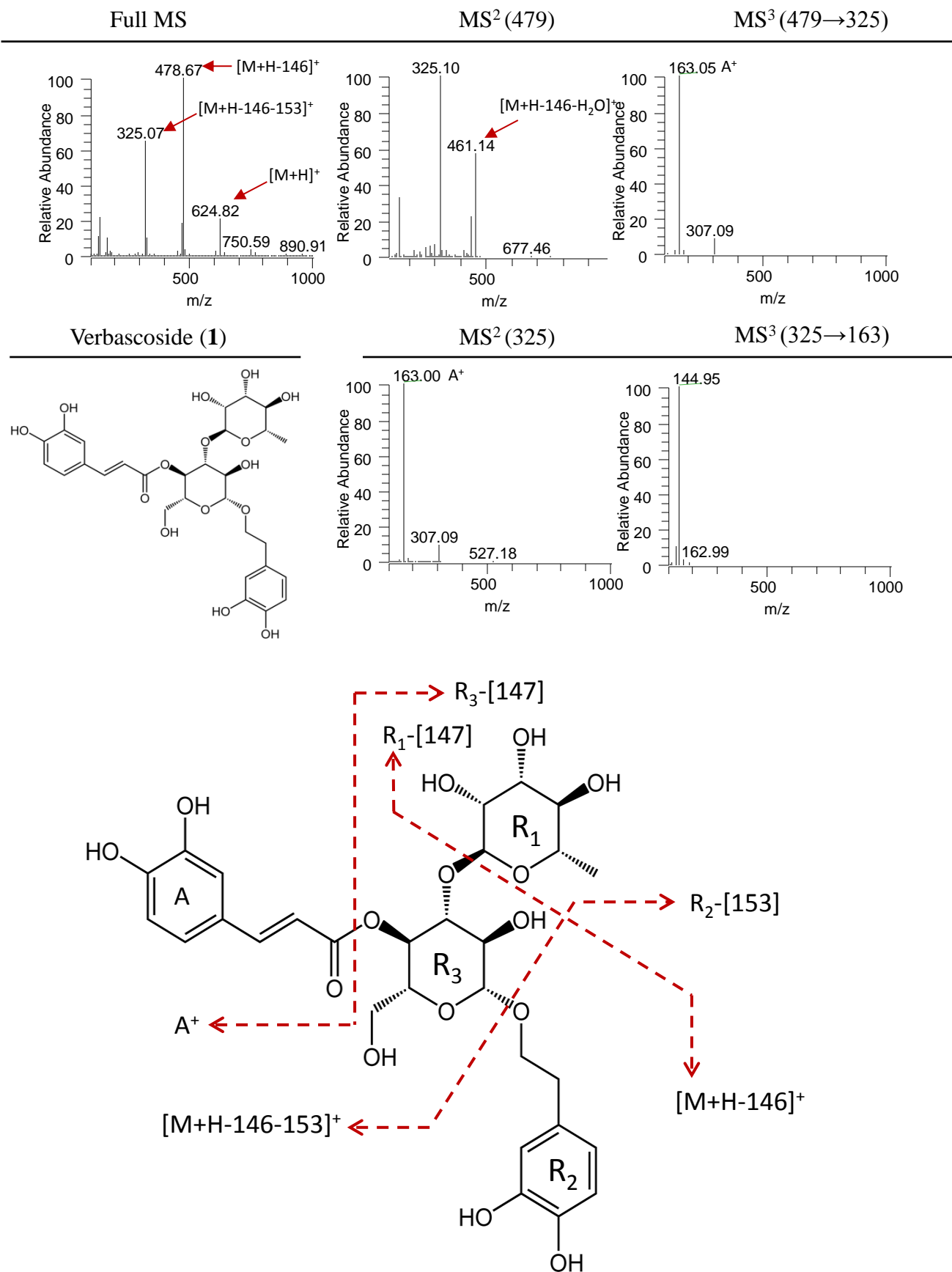


Figure S7. LC-mass spectrometry analyses of verbascoside (**1**). *Left:* Chemical structure. *Right:* Full MS scan and MSⁿ spectra under positive ion mode with an ESI source. *Below:* Fragmentation pattern.

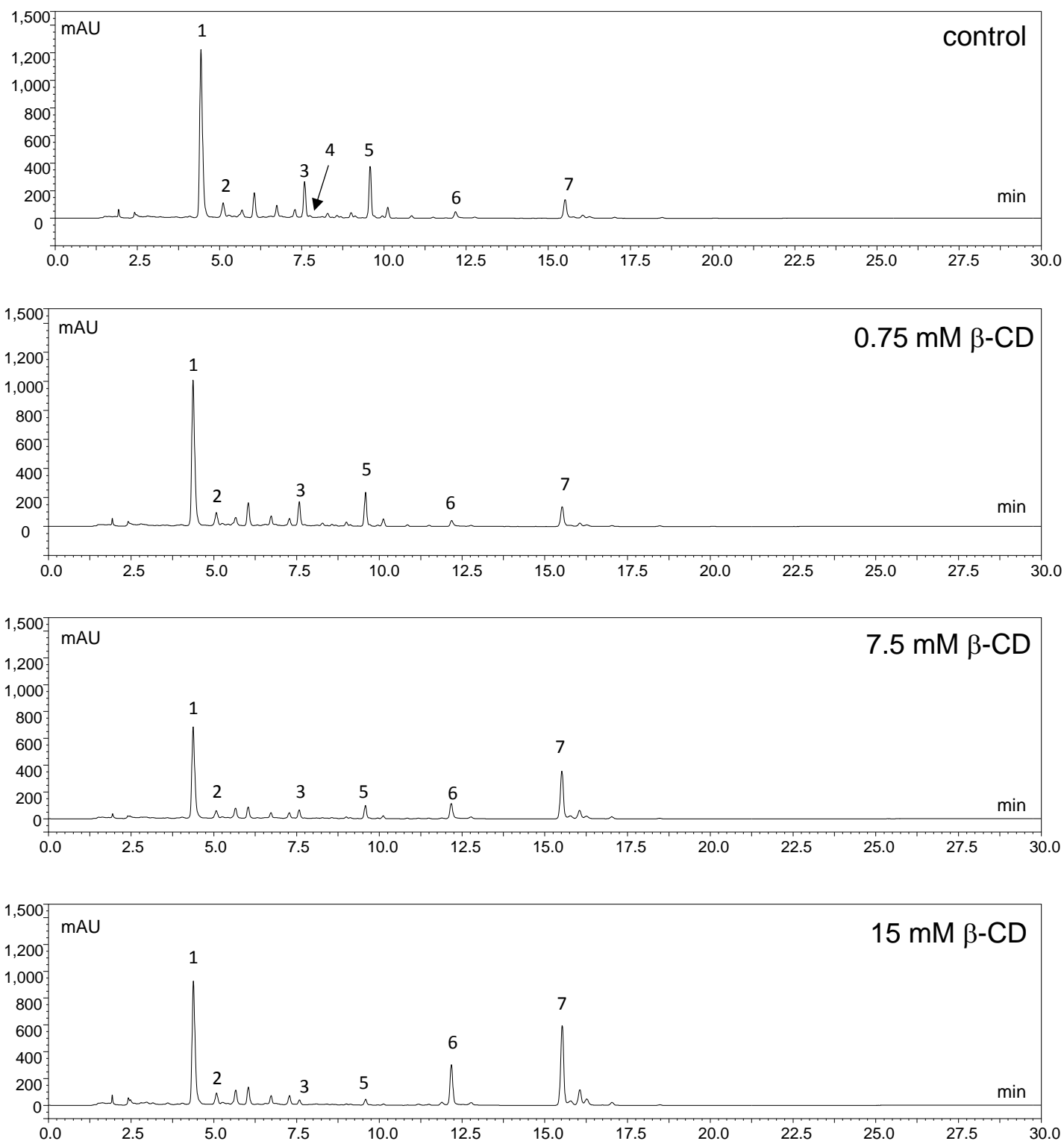


Figure S8. HPLC chromatograms (UV 277 nm) of methanol extracts from the tissue of hairy root cultures of *Scutellaria lateriflora* line 4 incubated under continuous light. Cultures were treated with methyl-β-cyclodextrin (β-CD) at day 30 for 24 h. **1**, Verbascoside; **2**, scutellarin; **3**, baicalin; **4**, scutellarein; **5**, wogonoside; **6**, baicalein; **7**, wogonin.

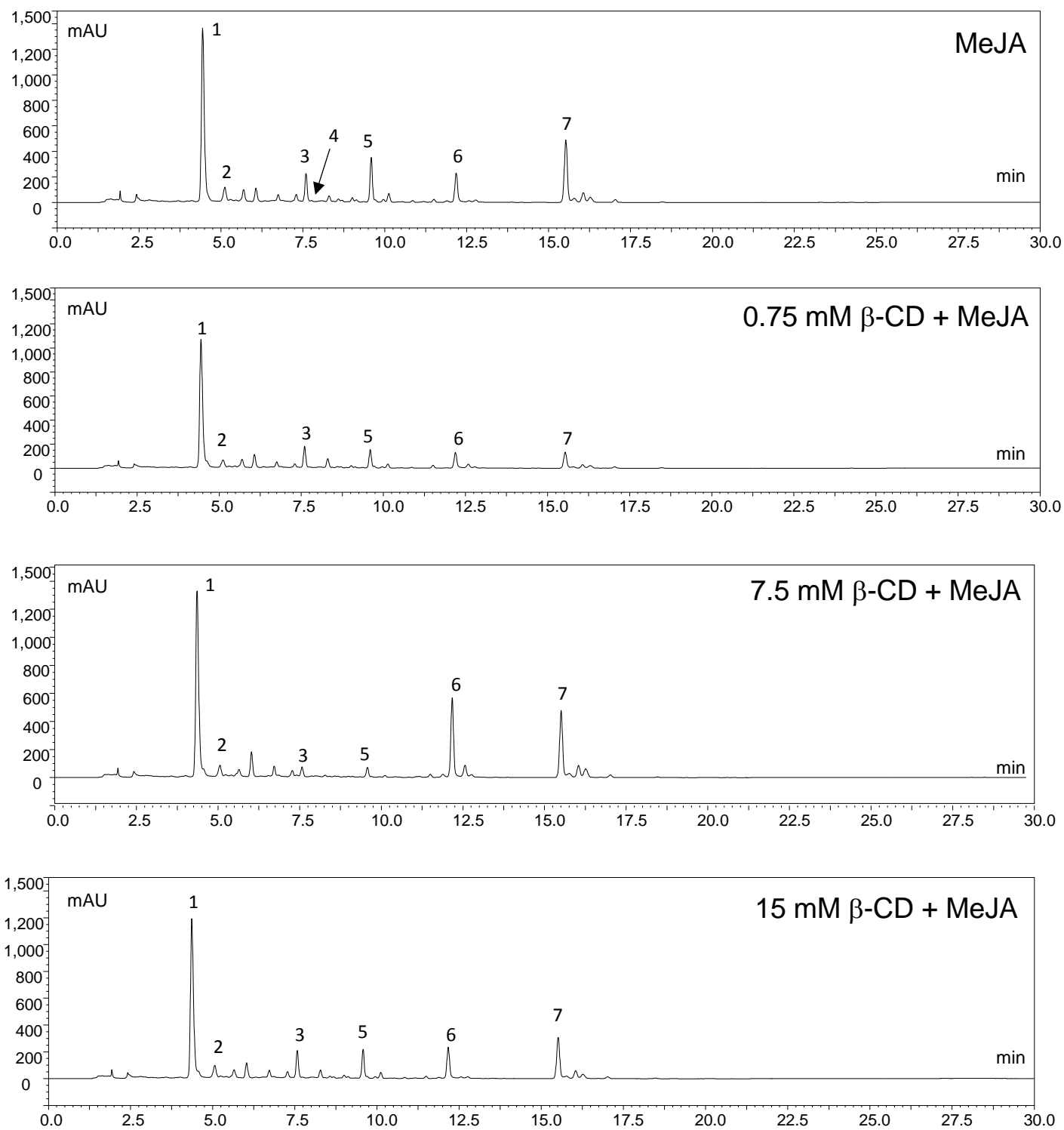


Figure S9. HPLC chromatograms (UV 277 nm) of methanol extracts from the tissue of hairy root cultures of *Scutellaria lateriflora* line 4 incubated under continuous light. Cultures were treated with methyl jasmonate (MeJA) alone or in combination with methyl- β -cyclodextrin (β -CD) at day 30 for 24 hours. **1**, Verbascoside; **2**, scutellarin; **3**, baicalin; **4**, scutellarein; **5**, wogonoside; **6**, baicalein; **7**, wogonin.

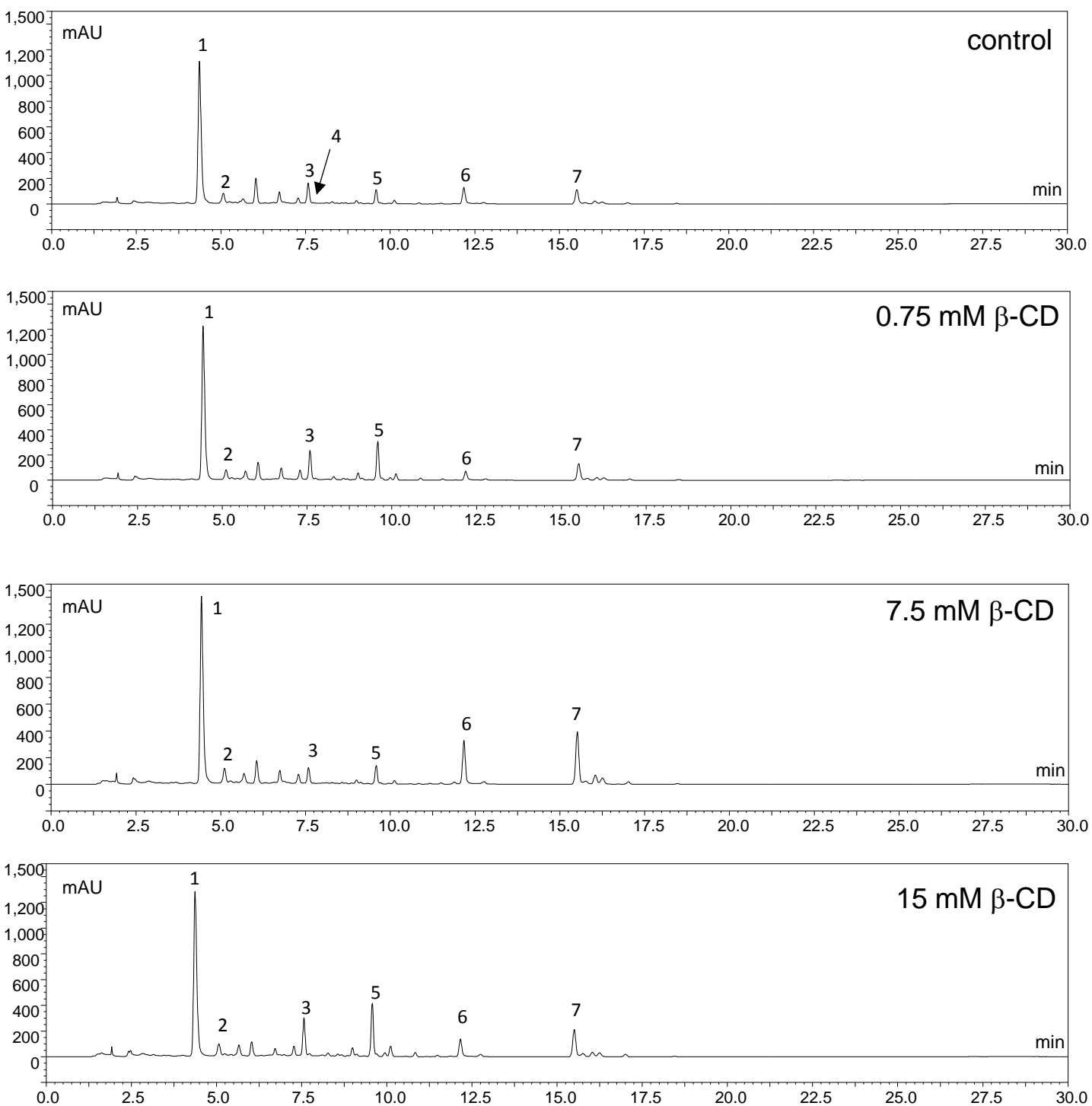


Figure S10. HPLC chromatograms (UV 277 nm) of methanol extracts from the tissue of hairy root cultures of *Scutellaria lateriflora* line 4 incubated under continuous darkness. Cultures were treated with methyl- β -cyclodextrin (β -CD) at day 30 for 24 hours. **1**, Verbascoside; **2**, scutellarin; **3**, baicalin; **4**, scutellarein; **5**, wogonoside; **6**, baicalein; **7**, wogonin.

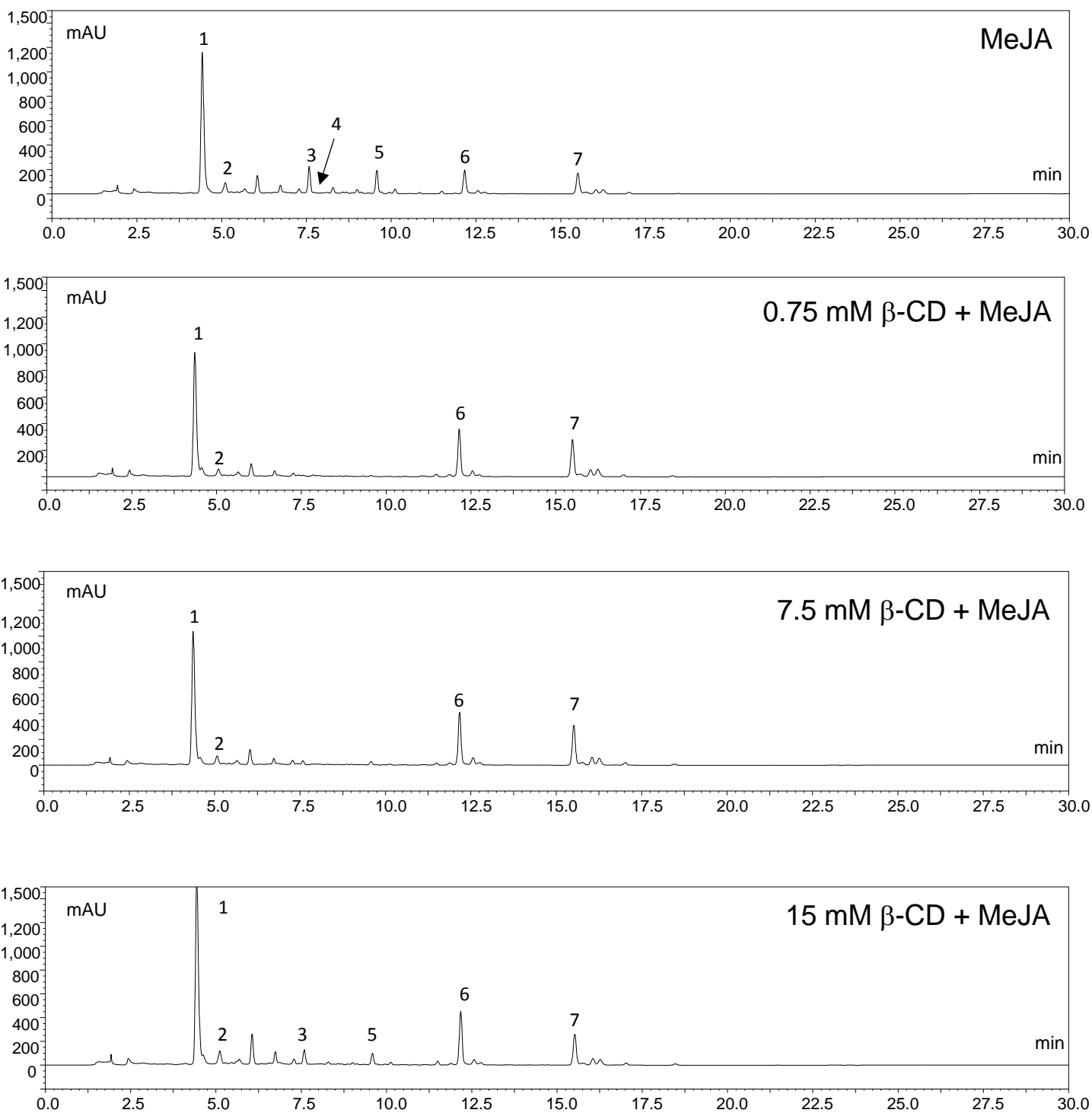


Figure S11. HPLC chromatograms (UV 277 nm) of methanol extracts from the tissue of hairy root cultures of *Scutellaria lateriflora* line 4 incubated under continuous darkness. Cultures were treated with methyl jasmonate (MeJA) alone or in combination with methyl- β -cyclodextrin (β -CD) at day 30 for 24 hours. **1**, Verbascoside; **2**, scutellarin; **3**, baicalin; **4**, scutellarein; **5**, wogonoside; **6**, baicalein; **7**, wogonin.

Table S1. Yield of phenolic compounds in the tissue of hairy root cultures of *Scutellaria lateriflora* line 4 incubated under continuous light and treated with different elicitors. Each value represents the average yield \pm standard deviation of three biological replicates. Yields are expressed in mg/g dry wt root. β -CD; methyl- β -cyclodextrin; MeJA; methyl jasmonate.

		β -CD				100 μ M MeJA + β -CD		
	Control	0.75 mM	7.5 mM	15 mM	100 μ M MeJA	0.75 mM	7.5 mM	15 mM
Verbascoside (1)	9.584 \pm 4.11	11.88 \pm 1.12	8.057 \pm 3.03	10.04 \pm 2.48	12.974 \pm 2.81	12.225 \pm 3.74	13.054 \pm 6.10	13.797 \pm 1.78
Scutellarin (2)	0.233 \pm 0.09	0.362 \pm 0.06	0.211 \pm 0.09	0.254 \pm 0.09	0.329 \pm 0.11	0.332 \pm 0.06	0.290 \pm 0.12	0.377 \pm 0.13
Scutellarein (3)	0.033 \pm 0	0.010 \pm 0.005	0.005 \pm 0	0.030 \pm 0	0.009 \pm 0.002	0.011 \pm 0.012	0.016 \pm 0.007	0.003 \pm 0.004
Baicalin (4)	0.510 \pm 0.46	0.400 \pm 0.30	0.202 \pm 0.20	0.071 \pm 0.04	0.435 \pm 0.25	0.515 \pm 0.23	0.397 \pm 0.45	0.510 \pm 0.26
Baicalein (5)	3.635 \pm 2.80	2.194 \pm 2.69	1.955 \pm 0.75	5.712 \pm 0.56	3.615 \pm 1.50	3.272 \pm 2.27	4.88 \pm 5.21	3.481 \pm 1.19
Wogonoside (6)	0.317 \pm 0.37	0.412 \pm 0.29	0.207 \pm 0.14	0.089 \pm 0.05	0.432 \pm 0.30	0.338 \pm 0.26	0.350 \pm 0.45	0.330 \pm 0.18
Wogonin (7)	0.557 \pm 0.42	0.322 \pm 0.44	0.352 \pm 0.17	0.751 \pm 0.15	0.479 \pm 0.21	0.274 \pm 0.16	0.284 \pm 0.35	0.334 \pm 0.06

Table S2. Yield of phenolic compounds in the tissue of hairy root cultures of *Scutellaria lateriflora* line 4 incubated under continuous dark and treated with different elicitors. Each value represents the average yield \pm standard deviation of three biological replicates. Yields are expressed in mg/g dry wt root. β -CD; methyl- β -cyclodextrin; MeJA; methyl jasmonate.

		β -CD				β -CD + 100 μ M MeJA		
	Control	0.75 mM	7.5 mM	15 mM	100 μ M MeJA	0.75 mM	7.5 mM	15 mM
Verbascoside (1)	14.481 \pm 1.65	11.423 \pm 2.81	13.608 \pm 3.11	16.462 \pm 1.14	16.191 \pm 3.91	10.725 \pm 1.18	11.121 \pm 3.84	15.537 \pm 2.87
Scutellarin (2)	0.315 \pm 0.07	0.228 \pm 0.06	0.315 \pm 0.09	0.453 \pm 0.01	0.356 \pm 0.05	0.230 \pm 0.04	0.211 \pm 0.02	0.355 \pm 0.16
Scutellarein (3)	0.010 \pm 0.01	0.009 \pm 0.01	0.005 \pm 0	0.022 \pm 0.02	0.013 \pm 0.01	0.017 \pm 0.01	0.003 \pm 0	0.036 \pm 0.04
Baicalin (4)	0.558 \pm 0.14	0.337 \pm 0.34	0.361 \pm 0.21	0.867 \pm 0.33	0.698 \pm 0.36	0.173 \pm 0.15	0.101 \pm 0.12	0.363 \pm 0.32
Baicalein (5)	0.242 \pm 0.13	0.282 \pm 0.32	0.427 \pm 0.20	0.186 \pm 0.15	0.449 \pm 0.29	0.688 \pm 0.20	0.606 \pm 0.26	0.670 \pm 0.36
Wogonoside (6)	0.335 \pm 0.14	0.253 \pm 0.29	0.212 \pm 0.08	0.515 \pm 0.25	0.360 \pm 0.18	0.099 \pm 0.08	0.019 \pm 0.02	0.227 \pm 0.16
Wogonin (7)	0.203 \pm 0.25	0.162 \pm 0.17	0.162 \pm 0.26	0.218 \pm 0.17	0.212 \pm 0.19	0.386 \pm 0.18	0.191 \pm 0.18	0.253 \pm 0.15