Supplementary materials

Antioxidant activities of phenolic metabolites from *Flemingia philippinensis* and application to DNA damage protection

Jeong Yoon Kim¹, Yan Wang², Yeong Hun Song¹, Zia Uddin¹,

Zuo Peng Li¹, Yeong Jun Ban¹ and Ki Hun Park^{1,*}

¹ Division of Applied Life Science (BK21 plus), IALS, Gyeongsang National University, Jinju 52828, Republic of Korea

² College of Food and Biological Engineering, Qiqihar University, Quqihar 161006, China

List of supporting information

- Figure S1. ¹H-NMR and ¹³C-NMR spectrums of compound 1
- Figure S2. EIMS and HREIMS data of compound 1
- Figure S3. ¹H-NMR and ¹³C-NMR spectrums of compound 2
- Figure S4. EIMS and HREIMS data of compound 2
- Figure S5. ¹H-NMR and ¹³C-NMR spectrums of compound 3
- Figure S6. EIMS and HREIMS data of compound 3
- Figure S7. ¹H-NMR and ¹³C-NMR spectrums of compound 4
- Figure S8. EIMS and HREIMS data of compound 4
- Figure S9. ¹H-NMR and ¹³C-NMR spectrums of compound 5
- Figure S10. EIMS and HREIMS data of compound 5
- Figure S11. ¹H-NMR and ¹³C-NMR spectrums of compound 6
- Figure S12. EIMS and HREIMS data of compound 6
- Figure S13. ¹H-NMR and ¹³C-NMR spectrums of compound 7
- Figure S14. EIMS and HREIMS data of compound 7
- Figure S15. ¹H-NMR and ¹³C-NMR spectrums of compound 8
- Figure S16. EIMS and HREIMS data of compound 8
- Figure S17. ¹H-NMR and ¹³C-NMR spectrums of compound 9
- Figure S18. EIMS and HREIMS data of compound 9
- Figure S19. ¹H-NMR and ¹³C-NMR spectrums of compound 10
- Figure S20. EIMS and HREIMS data of compound 10
- Figure S21. ¹H-NMR and ¹³C-NMR spectrums of compound 11
- Figure S22. EIMS and HREIMS data of compound 11
- Figure S23. ¹H-NMR and ¹³C-NMR spectrums of compound 12

- Figure S24. EIMS and HREIMS data of compound 12
- Figure S25. ¹H-NMR and ¹³C-NMR spectrums of compound 13
- Figure S26. EIMS and HREIMS data of compound 13
- Figure S27. ¹H-NMR and ¹³C-NMR spectrums of compound 14
- Figure S28. EIMS and HREIMS data of compound 14
- Figure S29. ¹H-NMR and ¹³C-NMR spectrums of compound 15
- Figure S30. EIMS and HREIMS data of compound 15
- Figure S31. ¹H-NMR and ¹³C-NMR spectrums of compound 16
- Figure S32. EIMS and HREIMS data of compound 16
- Figure S33. ¹H-NMR and ¹³C-NMR spectrums of compound 17
- Figure S34. EIMS and HREIMS data of compound 17
- Figure S35. ¹H-NMR and ¹³C-NMR spectrums of compound 18
- Figure S36. EIMS and HREIMS data of compound 18
- Figure S37. ESR spectra of DPPH radical scavenging effect of compounds1-9
- Figure S38. ESR spectra of DPPH radical scavenging effect of compounds10-18
- Figure S39. ESR spectra of hydroxyl radical scavenging effect of compounds1-9
- Figure S40. ESR spectra of hydroxyl radical scavenging effect of compounds10-18
- Figure S41. ESR spectra of superoxide radical scavenging effect of compounds1-9
- Figure S42. ESR spectra of superoxide radical scavenging effect of compounds10-18
- Figure S43-45. Results of electrophoresis by pBR322 plasmid DNA band intensity
- Table S1. pBR322 plasmid DNA damage protective effect of 1-18.
- Table S2. Protective effect of pBR322 plasmid DNA by dose dependent of compound 2



Figure 1. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 1(CD₃OD)



Figure 2. EIMS and HREIMS data of compound 1



Figure 3. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 2 (CDCl₃)



Figure 4. EIMS and HREIMS data of compound 2



Figure 5. ¹H (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 3 (Acetone-*d*₆)



Figure 6. EIMS and HREIMS data of compound 3





Figure 7. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 4 (CDCl₃)



Figure 8. EIMS and HREIMS data of compound 4



Figure 9. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 5 (Acetone-d)



Figure 10. EIMS and HREIMS data of compound 5



Figure 11. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 6 (CDCl₃)



Figure 12. EIMS and HREIMS data of compound 6



Figure 13. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 7 (CDCl₃)



Figure 14. EIMS and HREIMS data of compound 7



Figure 15. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 8 (CDCl₃)



Figure 16. EIMS and HREIMS data of compound 8



Figure 17. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound 9 (CDCl₃)



Figure 18. EIMS and HREIMS data of compound 9



Figure 19. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums of compound **10** (Acetone- d_6)



Figure 20. EIMS and HREIMS data of compound 10



Figure 21. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

of compound 11 (CDCl₃)



Figure 22. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

of compound 11 (CDCl₃)



Figure 23. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

of compound 12 (CDCl₃)



Figure 24. EIMS and HREIMS data of compound 12



of compound **13** (CDCl₃)



Figure 26. EIMS and HREIMS data of compound 13



Figure 27. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

of compound $14\ (\text{CDCl}_3)$



Figure 28. EIMS and HREIMS data of compound 14



Figure 29. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

of compound 15 (CDCl₃)



Figure 30. EIMS and HREIMS data of compound 15



Figure 31. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

of compound 16 (CDCl₃)



Figure 32. EIMS and HREIMS data of compound 16



Figure 33. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

90 80 70 60

50

40 30 20

110 100 f1 (ppm)

140 130 120

200 190 180 170 160 150

-0,0E+00 . --5,0E+07

10 0

of compound 17 (CDCl₃)



Figure 34. EIMS and HREIMS data of compound 17



Figure 35. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectrums

of compound 18 (CDCl₃)



Figure 36. EIMS and HREIMS data of compound 18



Figure 37. ESR spectra of DPPH radical scavenging effect of compounds1-9 (7.0 mg/ml)



Figure 38. ESR spectra of DPPH radical scavenging effect of compounds10-18 (7.0 mg/ml)



Figure 39. ESR spectra of hydroxyl radical scavenging effect of compounds1-9 (7.5 mg/ml)



Figure 40. ESR spectra of hydroxyl radical scavenging effect of compounds**10-18** (7.5 mg/ml)



Figure 41. ESR spectra of superoxide radical scavenging effect of compounds1-9 (25 mg/ml)



Figure 42. ESR spectra of superoxide radical scavenging effect of compounds10-18 (25 mg/ml)



Lane And Band Analysis











Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		N/A	0.214	2,155,794	N/A	1.02	89.3	82.4
2		N/A	0.655	215,812	N/A	0.12	10.7	9.9





 Band No.
 Band Label
 Mol. Wt. (KDa)
 Relative Front
 Volume (Int)
 Abs. Quant.
 Rel. Quant.
 Band %
 Lane %

 1
 N/A
 0.202
 284,152
 N/A
 0.13
 10.4
 7.2

 2
 N/A
 0.738
 1,471,044
 N/A
 0.83
 87.5
 88.2



Band No.	Band Label	Mol. Wt. (I	KDa)	Relative Fron	Volume	(Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1			N/A	0.16	1,767	,252	N/A	1.00	77.1	67.8
2			N/A	0.60	399	,000	N/A	0.30	22.9	20.1





Band No. Band Label	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0.202	226,134	N/A	0.13	15.3	15.0
2	N/A	0.702	1,251,642	N/A	0.71	84.7	83.1





Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		N/A	0.310	1,998,383	N/A	0.79	87.5	82.5
2		N/A	0.726	198,798	N/A	0.11	12.5	11.8



Figure 43. Results of electrophoresis by pBR322 plasmid DNA band intensity; Lane 1, pBR322 plasmid DNA; Lane 2, oxidative DNA; Lane 3, compound 1; Lane 4, compound 2; Lane 5, compound 3; Lane 6, compound 4; Lane 7, compound 5; Lane 8, compound 6; Lane 9, compound 7; Lane 10, compound 8; Lane 11, compound 9.



























 Band No.
 Band Label
 Mol. WL (KDa)
 Relative Front
 Volume (Int)
 Abs. Quant.
 Rel. Quant.
 Band %
 Lane %

 1
 N/A
 0.096
 430,499
 N/A
 0.005
 7.6
 7.1

 2
 N/A
 0.904
 1,619,940
 N/A
 0.66
 92.4
 85.9

Lane 6



 Band No.
 Band Label Mol. Wt. (KDa)
 Relative Front
 Volume (Int)
 Abs. Quant.
 Rel. Quant.
 Band %
 Lane %

 1
 N/A
 0.144
 1.273,941
 N/A
 0.52
 49.2
 46.6

 2
 N/A
 0.875
 1.315,875
 N/A
 0.53
 50.8
 48.1

Lane 8





Lane 10



Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		N/A	0.173	2,300,434	N/A	0.61	88.1	82.1
2		N/A	0.788	204,798	N/A	0.08	11.9	11.1



Figure 44. Results of electrophoresis by pBR322 plasmid DNA band intensity; Lane 3, compound 10; Lane 4, compound 11; Lane 5, compound 12; Lane 6, compound 13; Lane 7, compound 14; Lane 8, compound 15; Lane 9, compound 16; Lane 10, compound 17; Lane 11, compound 18; Lane 12, trolox.





Figure 45. Results of electrophoresis by pBR322 plasmid DNA band intensity against dosedependent with compound **2**; Lane 1, pBR322 plasmid DNA (control); Lane 2, oxidative DNA; Lane 3, 7.5 μ M; Lane 4, 15.0 μ M; Lane 5, 30.0 μ M; Lane 6, 60.0 μ M.

	scDNA band intensity	ocDNA band intensity	DNA damage (%)	DNA protective effect (%)
pBR322 DNA	2472.2	77.3	-	_
oxidative DNA	399.2	1767.2	-	-
1	215.8	2155.8	87.2	12.8
2	1251.6	226.1	9.1	90.9
3	1471.0	284.15	11.5	88.5
4	198.8	1998.4	80.8	19.2
5	858.7	1133.4	45.8	54.2
6	1250.2	383.1	15.5	84.5
7	793.5	1504.5	60.9	39.1
8	1067.2	1789.9	72.4	27.6
9	1115.1	1800.6	72.8	27.2
10	1648.9	384.9	15.6	84.4
11	1619.9	430.5	17.4	82.6
12	1074.9	914.2	37.0	63.0
13	1315.9	1273.9	51.5	48.5
14	1462.6	1218.6	49.3	50.7
15	1919.8	532.2	21.5	78.5
16	2153.5	505.5	20.4	79.6
17	204.8	2300.5	93.1	6.9
18	190.8	1847.5	74.7	25.3
Trolox	213.2	2000.5	80.9	19.1

 Table 1. pBR322 plasmid DNA damage protective effect of 1-18.

	scDNA	ocDNA	DNA damage	DNA protective
	band intensity	band intensity	(%)	effect (%)
pBR322 DNA	2854.0	21.5	-	-
oxidative DNA	104.7	2488.2	-	-
7.5 μM	501.4	2125.4	74.5	25.5
15.0 μM	1033.2	1494.3	52.4	47.6
30.0 μM	1844.3	780.5	27.3	72.7
60.0 µM	2740.4	312.4	10.9	89.1

 Table 2. Protective effect of pBR322 plasmid DNA by dose dependent of compound 2