

Supplementary Materials

Analysis of Antioxidant Phytochemicals and Anti-Inflammatory Effect from *Vitex rotundifolia* L.f.

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1. Optimization of extraction time by sonification samples using 80% EtOH solvent

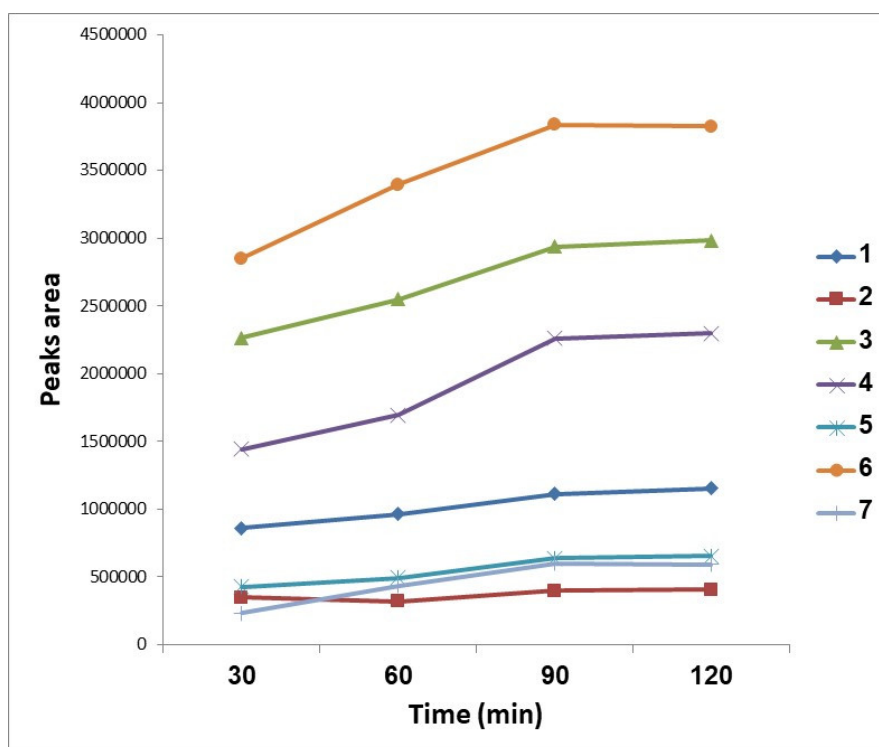


Figure S1. The amounts of analytes stimulation at different time.

2. Spectroscopic data of marker compounds (1–7):

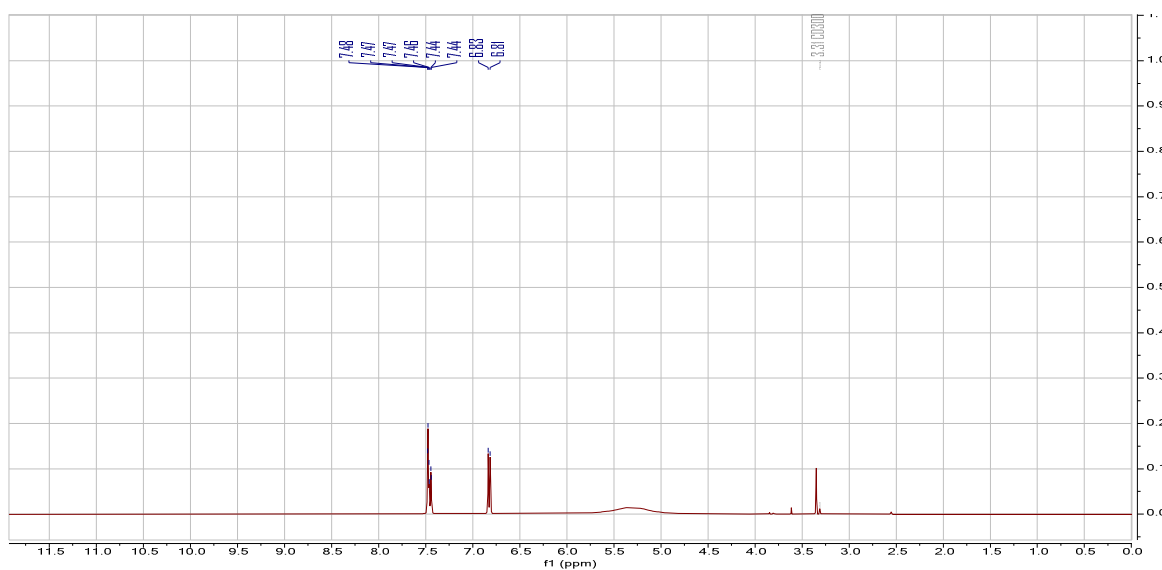


Figure S2. ¹H-NMR (400 MHz, CD₃OD) spectrum of compound 1.

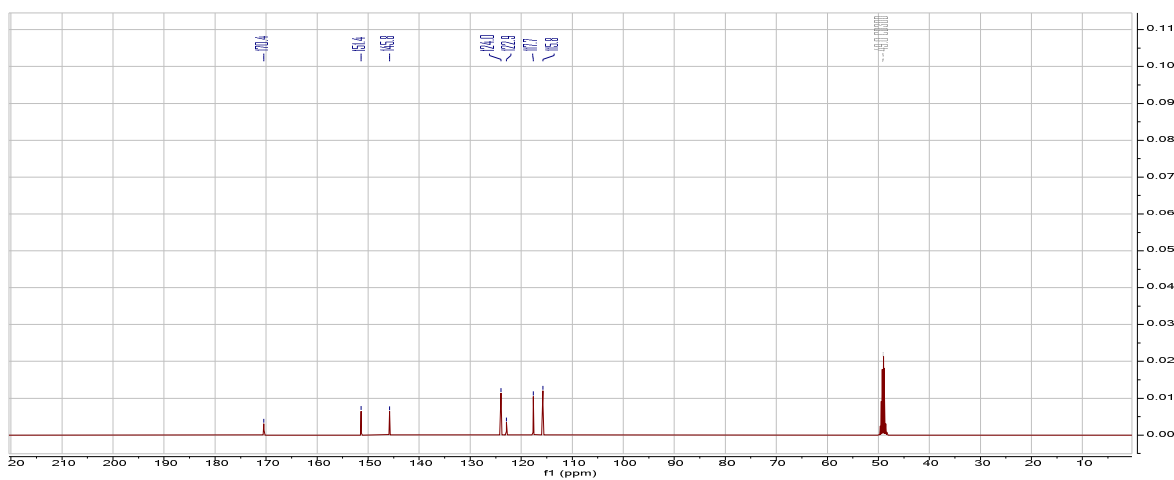


Figure S3. ¹³C-NMR (100 MHz, CD₃OD) spectrum of compound 1.

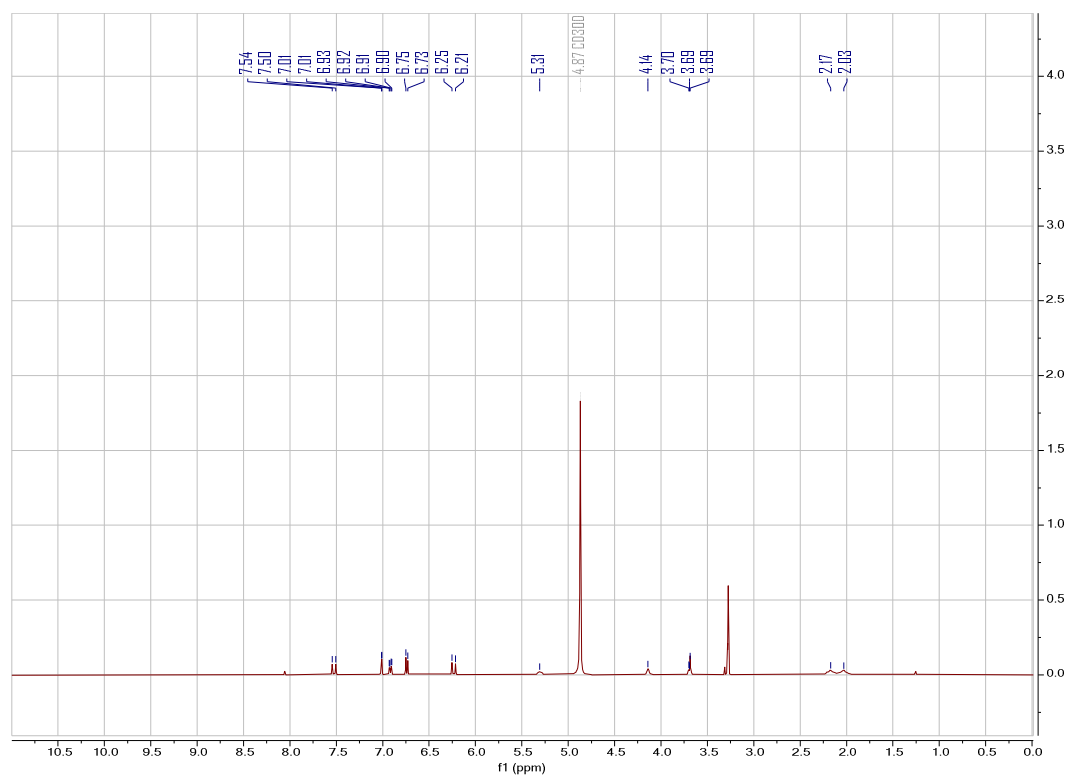


Figure S4. ^1H -NMR (400 MHz, CD_3OD) spectrum of compound **2**.

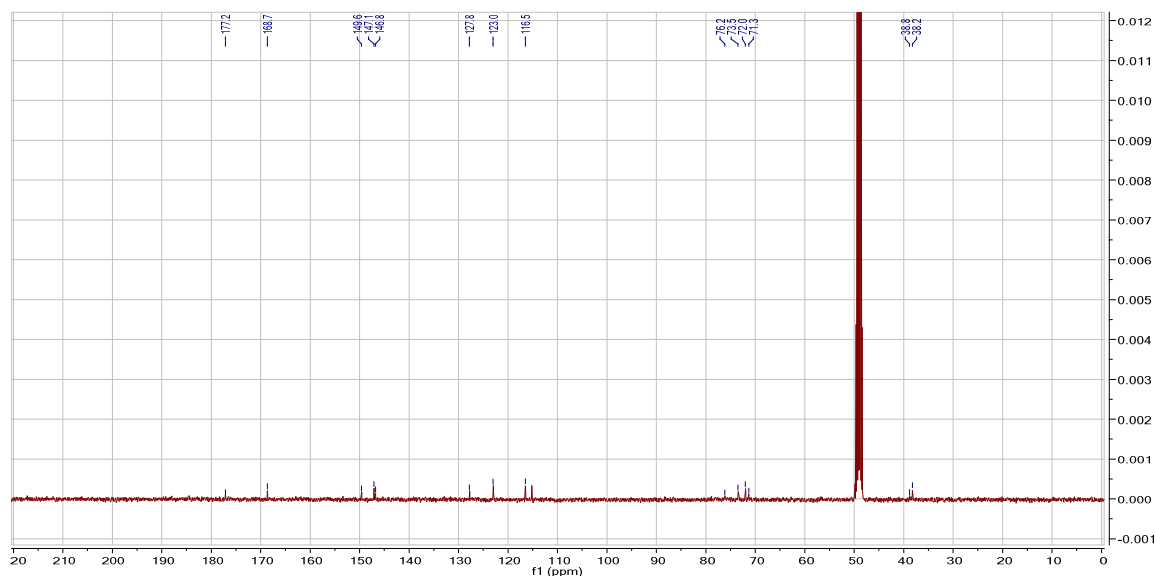


Figure S5. ^{13}C -NMR (100 MHz, CD_3OD) spectrum of compound **2**.

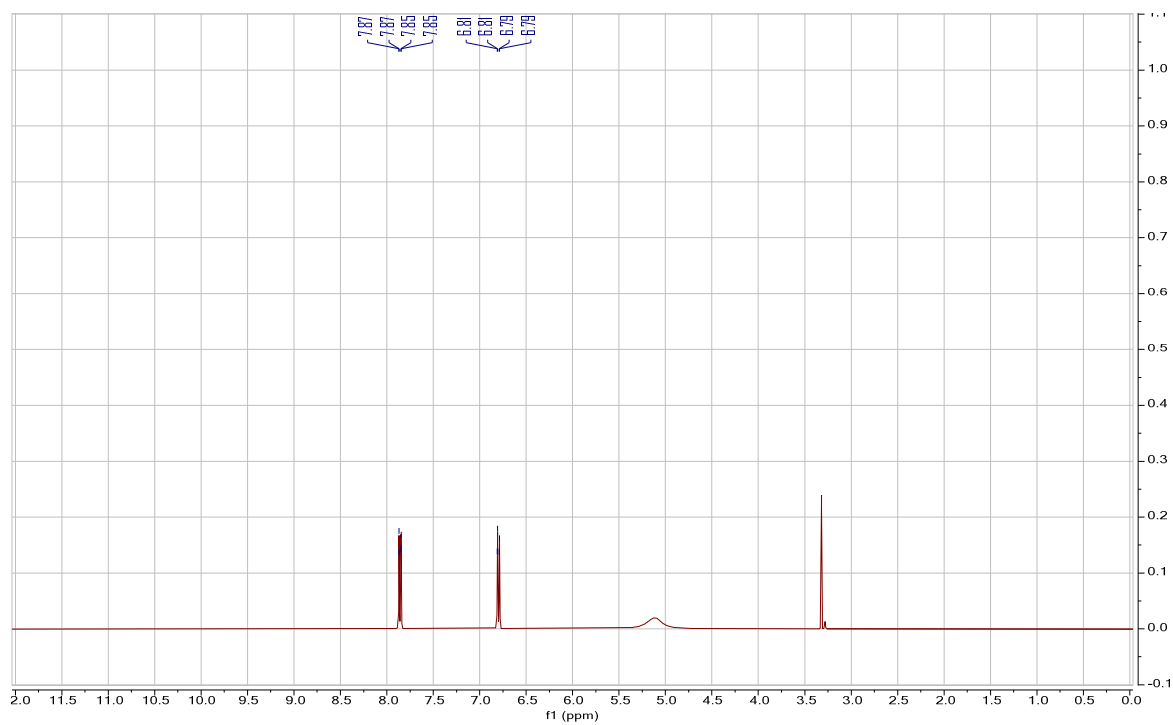


Figure S6. ¹H-NMR (400 MHz, CD₃OD) spectrum of compound 3.

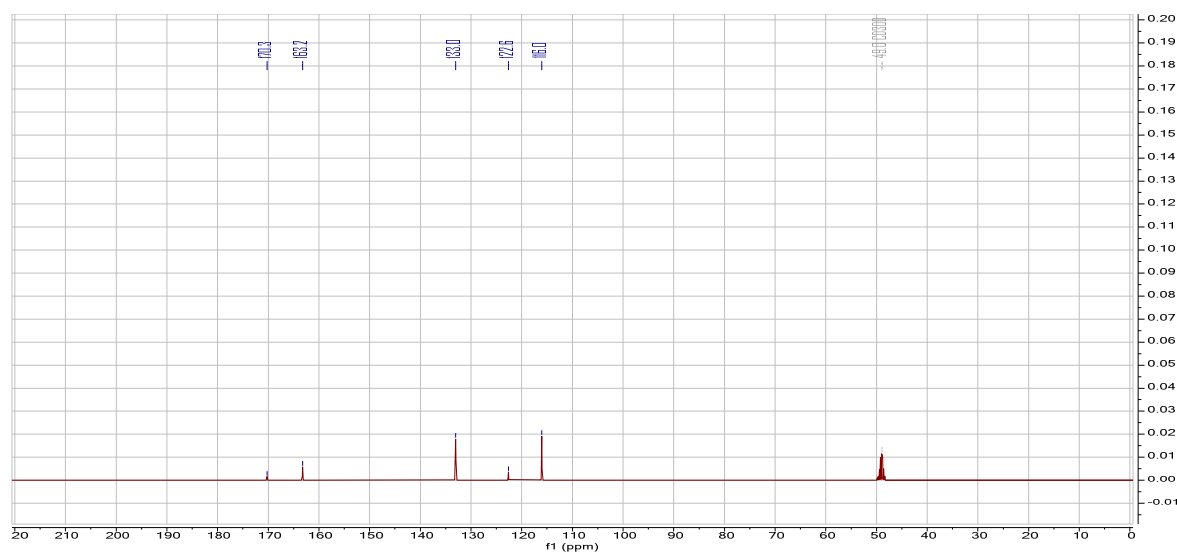


Figure S7. ¹³C-NMR (100 MHz, CD₃OD) spectrum of compound 3.

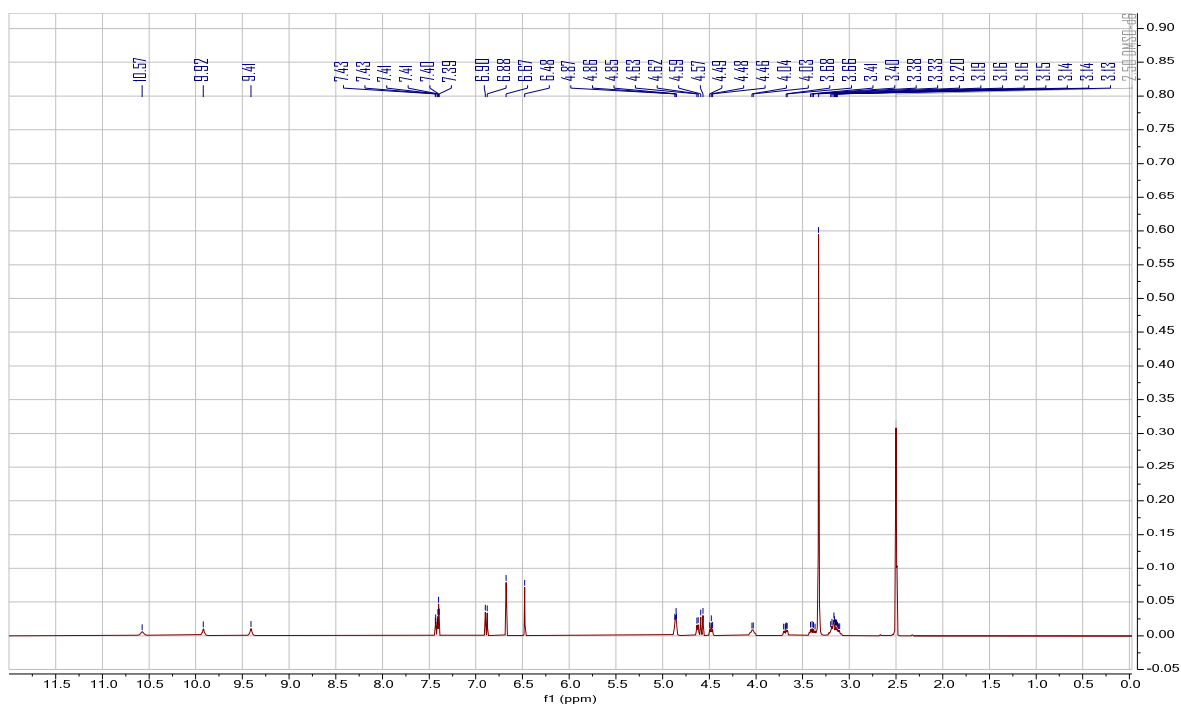


Figure S8. ^1H -NMR (400 MHz, CD_3OD) spectrum of compound **4**.

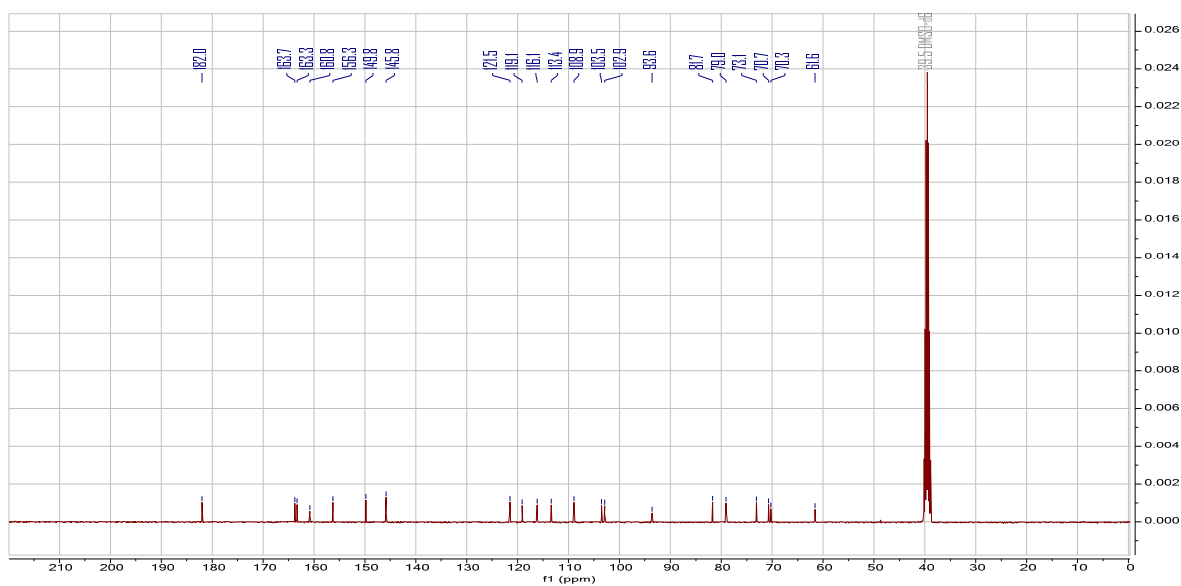


Figure S9. ^{13}C -NMR (100 MHz, CD_3OD) spectrum of compound **4**.

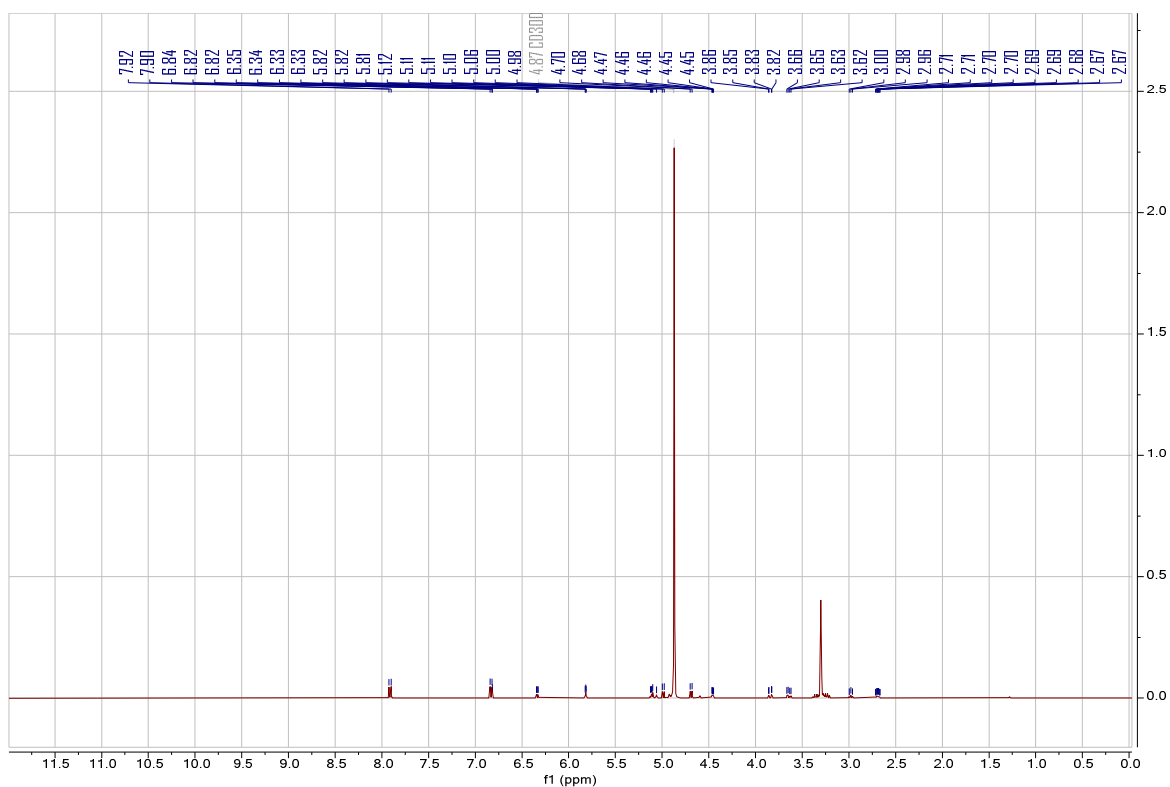


Figure S10. ¹H-NMR (400 MHz, CD₃OD) spectrum of compound 5.

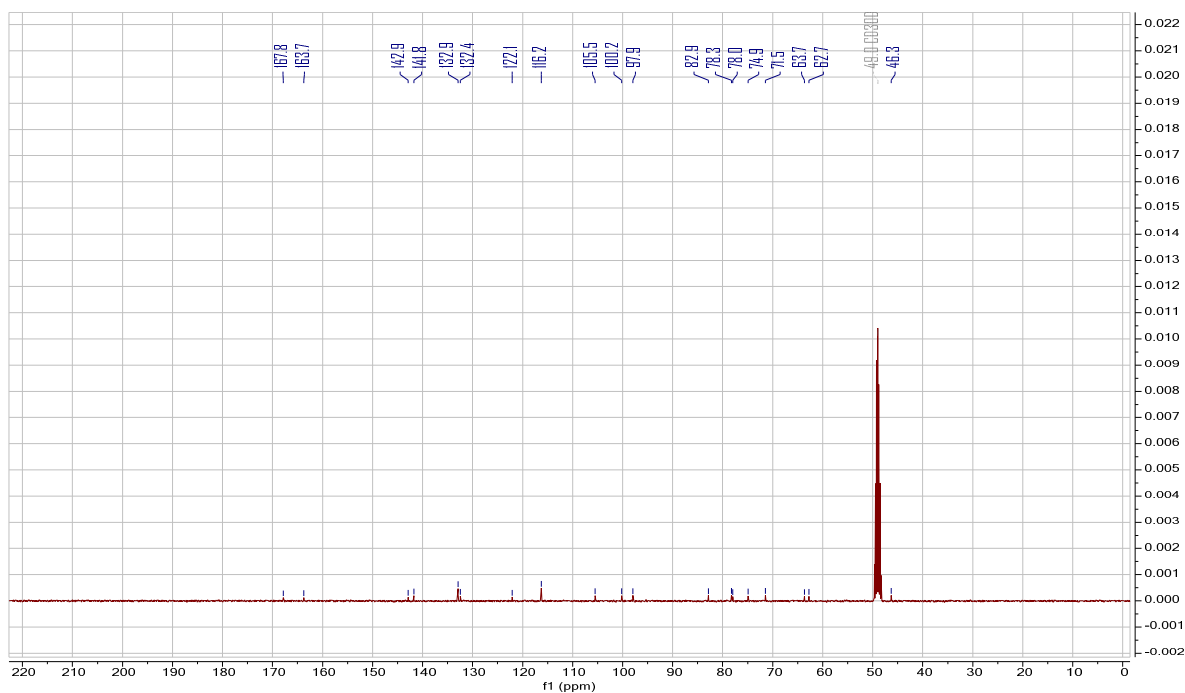


Figure S11. ¹³C-NMR (100 MHz, CD₃OD) spectrum of compound 5.

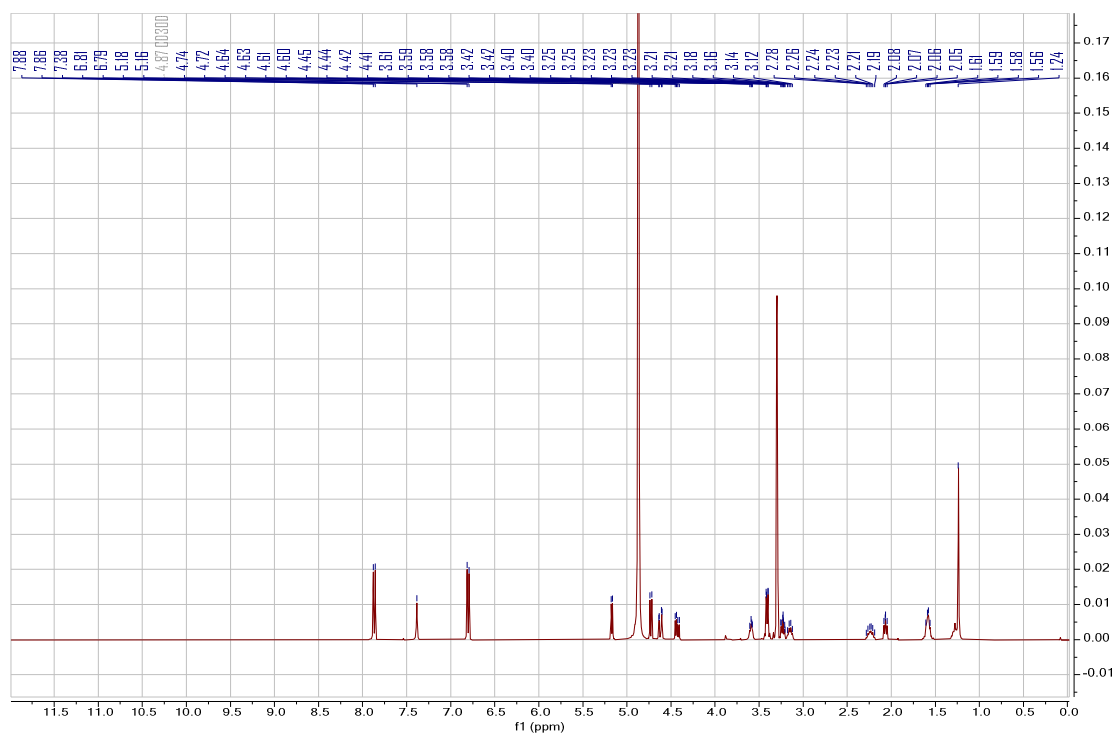


Figure S12. ^1H -NMR (400 MHz, CD_3OD) spectrum of compound **6**.

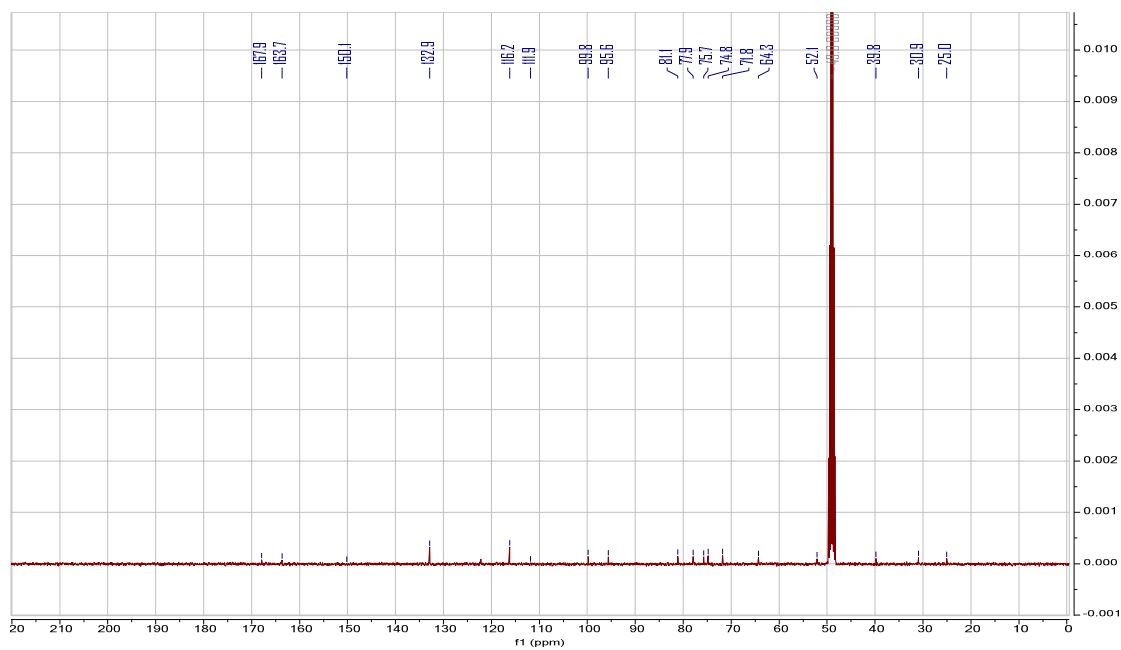


Figure S13. ^{13}C -NMR (100 MHz, CD_3OD) spectrum of compound **6**.

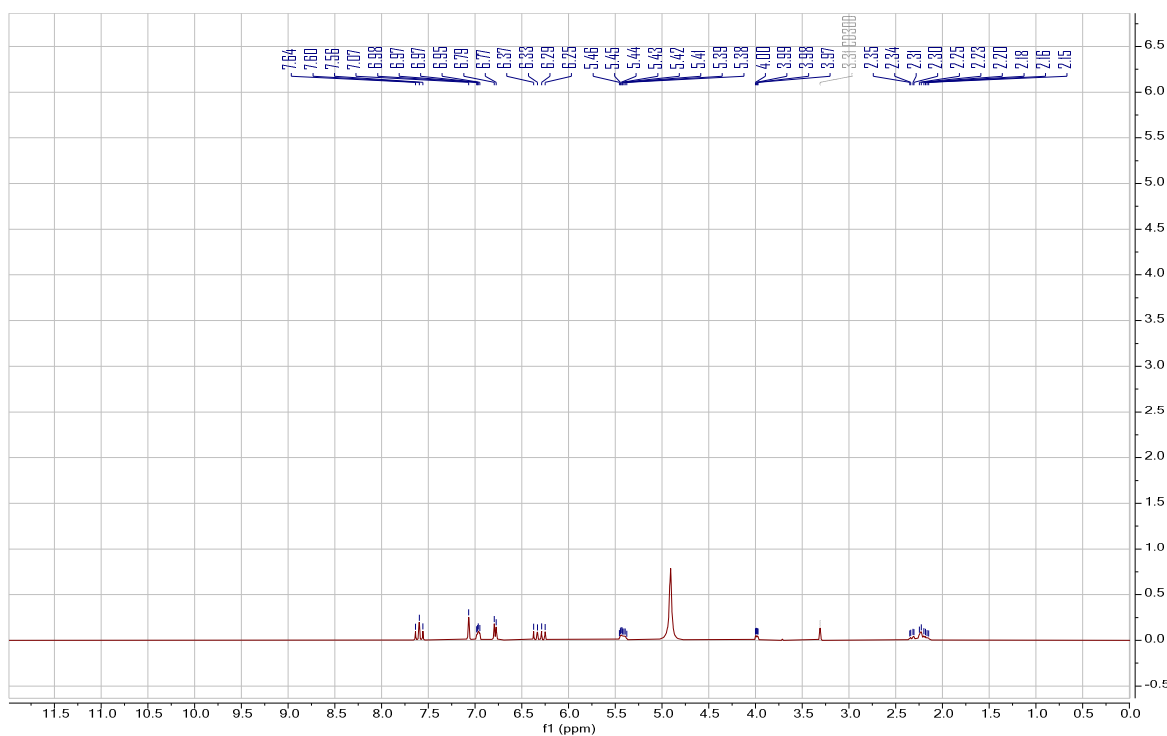


Figure S14. ^1H -NMR (400 MHz, CD_3OD) spectrum of compound 7.

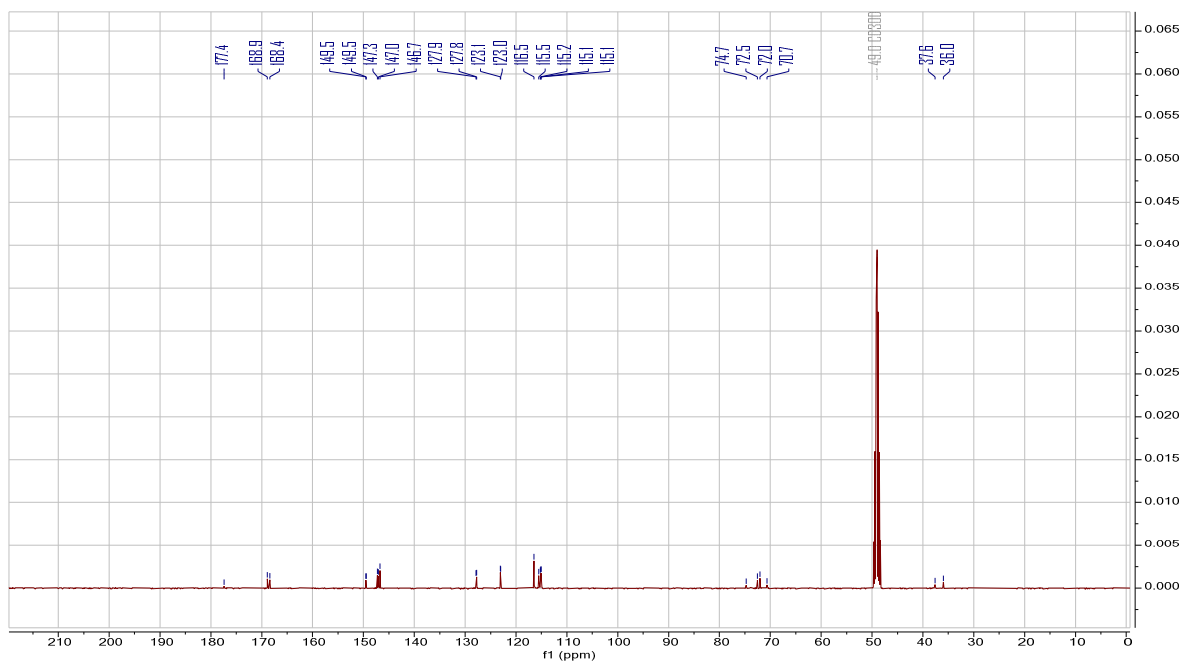


Figure S15. ^{13}C -NMR (100 MHz, CD_3OD) spectrum of compound 7.

3. Validation method:

Table S1. Retention time and purity of marker compounds (1–7).

Compound	Calibration curve	Regression Equation	RT (min)	Purity (%)	Correlation Coefficient (r^2)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Protocatechuic acid (1)	$0.98 \div 125$	$y = 32515x + 48307$	15.1	98.46	0.9995	0.24	0.74
Chlorogenic acid (2)	$0.98 \div 125$	$y = 10106x + 17177$	18.4	97.03	0.9992	0.32	1.18
4- <i>p</i> -Hydroxybenzoic acid (3)	$0.98 \div 125$	$y = 102986x + 168374$	19.4	98.01	0.9982	0.35	1.29
Orientin (4)	$12.5 \div 1600$	$y = 20055x + 13929$	22.2	98.04	0.9995	0.37	1.1
Agnuside (5)	$0.98 \div 500$	$y = 20256x + 23359$	23.0	97.58	0.9995	0.15	0.46
6'- <i>p</i> -Hydroxybenzoyl-mussaenosidic acid (6)	$0.95 \div 62.5$	$y = 45543x - 7338$	24.2	96.65	0.9984	0.22	0.69
3,5-Dicaffeoylquinic acid (7)	$0.95 \div 62.5$	$y = 25502x - 10971$	27.7	97.52	0.9995	0.25	0.77

4. Biological activities:

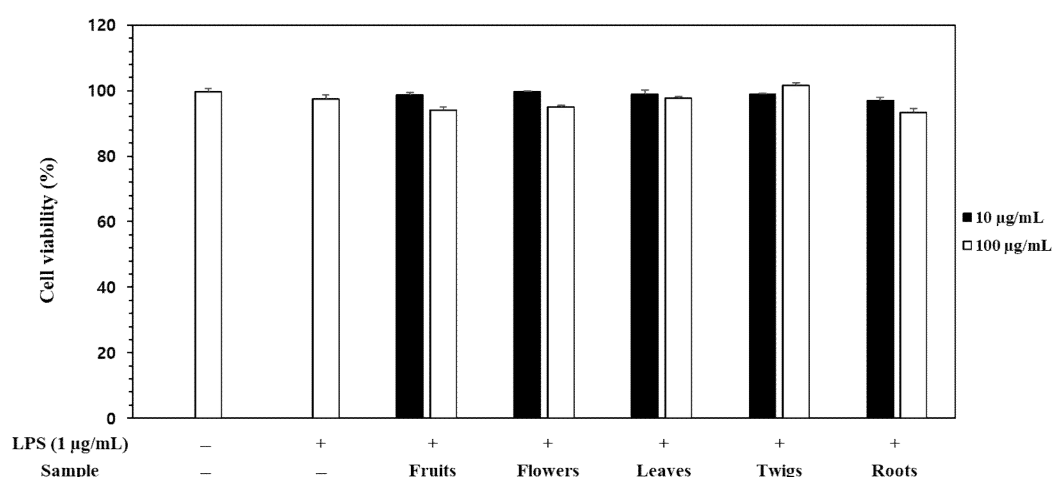


Figure S16. Cell viability of crude extracts on RAW264.7 cells. Negative and positive control were added with/without LPS-stimulation by treatment with medium. The cell viability was expressed as a percentage between the untreated control and treated sample cells. The data are expressed as the mean \pm SD ($n = 3$) of three individual experiments.

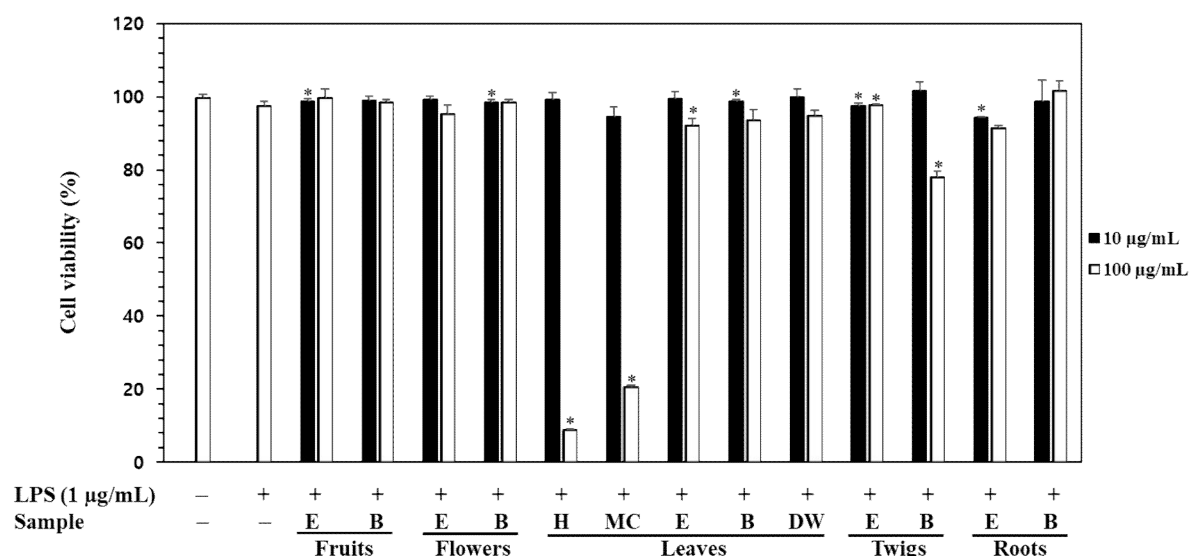


Figure S17. Cell viability of crude extracts and fractions on RAW264.7 cells. Negative and positive control were added with/without LPS-stimulation by treatment with medium. The cell viability was expressed as a percentage between the untreated control and treated sample cells. The data are expressed as the mean \pm SD ($n = 3$) of three individual experiments. * $p < 0.05$.

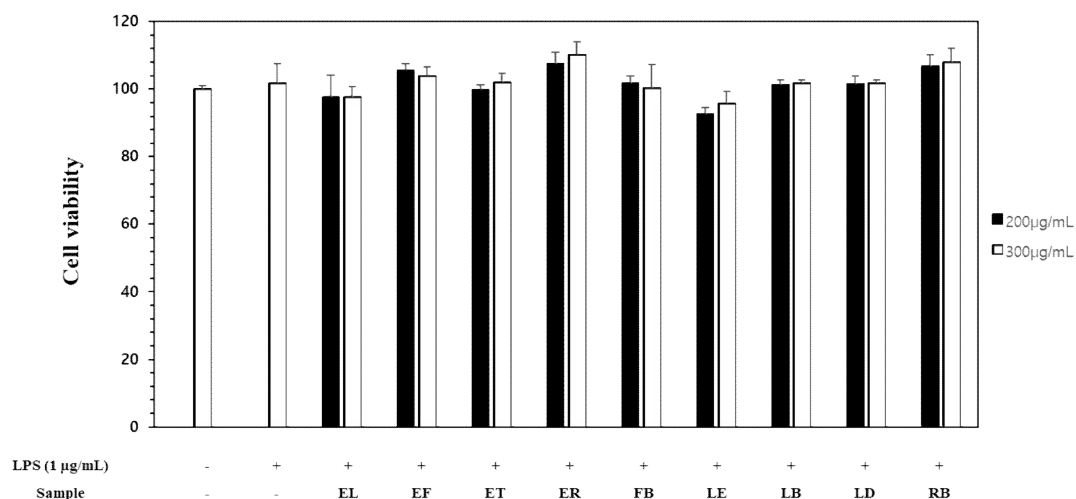


Figure S18. Cell viability of crude extracts and fractions on RAW264.7 cells. Negative and positive control were added with/without LPS-stimulation by treatment with medium. The cell viability was expressed as a percentage between the untreated control and treated sample cells. The data are expressed as the mean \pm SD ($n = 3$) of three individual experiments. $*p < 0.05$.