

## Anti-inflammatory activity of the constituents from the leaves of *Perilla frutense* var. *acuta*

Isoo Youn <sup>1,†</sup>, Sujin Han <sup>1,†</sup>, Hee Jin Jung <sup>2</sup>, Sang Gyun Noh <sup>2</sup>, Hae Young Chung <sup>2</sup>, Yeon Kyoung Koo <sup>3</sup>,

Sunhye Shin <sup>4,\*</sup>, and Eun Kyoung Seo <sup>4,\*</sup>

<sup>1</sup> Graduate School of Pharmaceutical Sciences, College of Pharmacy, Ewha Womans University, Seoul 03760, Korea; [isooyoun87@gmail.com](mailto:isooyoun87@gmail.com) (I.Y.); [sujinh94@gmail.com](mailto:sujinh94@gmail.com) (S.H.); [yuny@ewha.ac.kr](mailto:yuny@ewha.ac.kr) (E.K.S.)

<sup>2</sup> Department of Pharmacy, College of Pharmacy, Pusan National University, Busan 46241, Korea; [hjjung2046@pusan.ac.kr](mailto:hjjung2046@pusan.ac.kr) (H.J.J.); [rskrsk92@pusan.ac.kr](mailto:rskrsk92@pusan.ac.kr) (S.G.N.); [hyjung@pusan.ac.kr](mailto:hyjung@pusan.ac.kr) (H.Y.C.)

<sup>3</sup> Department of R&I Center, COSMAXBIO, Seongnam 13487, Korea; [ygkoo@cosmax.com](mailto:ygkoo@cosmax.com) (Y.K.K.)

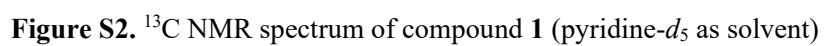
<sup>4</sup> Major of Food and Nutrition, Division of Applied Food System, Seoul Women's University, Seoul 01797, Korea; [ygkoo@cosmax.com](mailto:ygkoo@cosmax.com) (Y.K.K.)

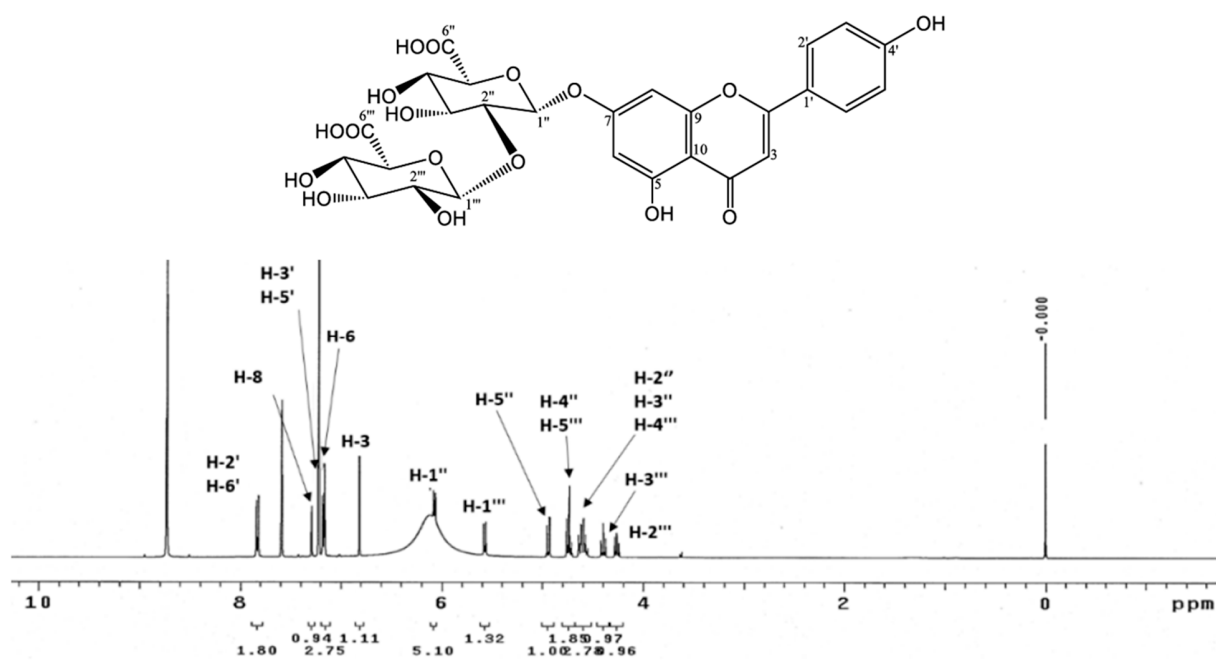
\* Correspondence: [yuny@ewha.ac.kr](mailto:yuny@ewha.ac.kr) (E.K.S.); [sshin@swu.ac.kr](mailto:sshin@swu.ac.kr) (S.S.)

† These authors contributed equally to this work.

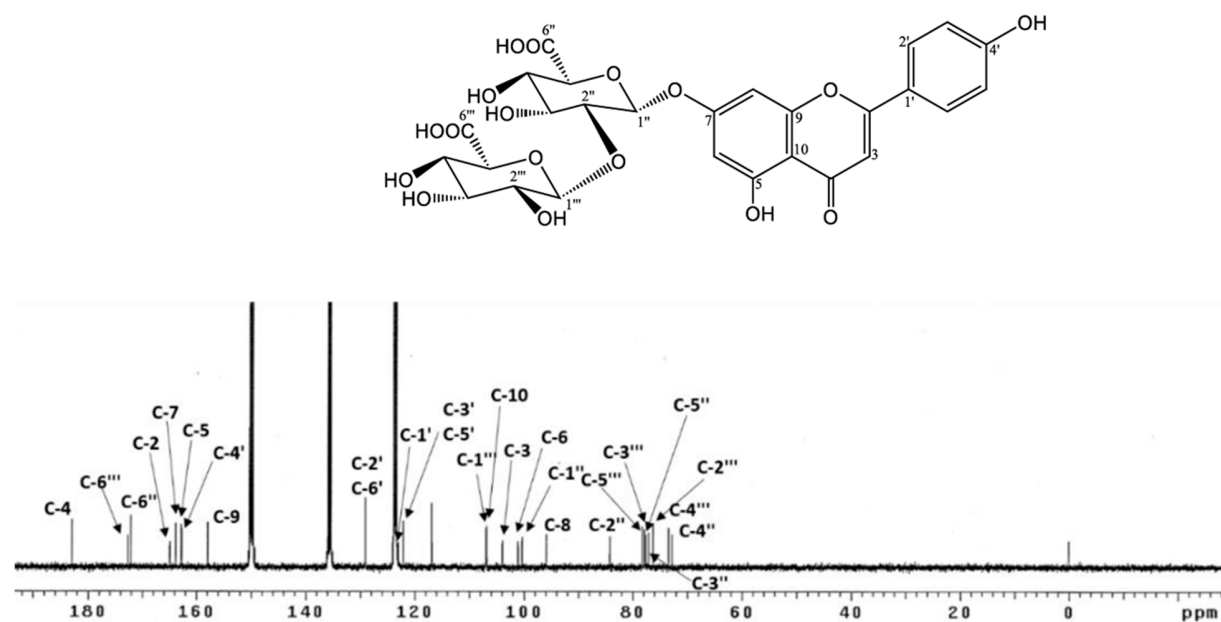
## TABLE OF CONTENTS

<b>Figure S1.</b> <sup>1</sup> H NMR spectrum of compound <b>1</b> .....	3
<b>Figure S2.</b> <sup>13</sup> C NMR spectrum of compound <b>1</b> .....	3
<b>Figure S3.</b> <sup>1</sup> H NMR spectrum of compound <b>2</b> .....	4
<b>Figure S4.</b> <sup>13</sup> C NMR spectrum of compound <b>2</b> .....	4
<b>Figure S5.</b> <sup>1</sup> H NMR spectrum of compound <b>3</b> .....	5
<b>Figure S6.</b> <sup>13</sup> C NMR spectrum of compound <b>3</b> .....	5
<b>Table S1.</b> Primer sequences used for quantitative real-time PCR.....	6
<b>Table S2.</b> The cell viability (%) of Raw 264.7 cells using an MTT assay.....	6
<b>Table S3.</b> PPAR-α agonistic potency of <b>1-3</b> compared to that of the control.....	6
<b>Table S4.</b> PPAR-δ agonistic potency of <b>1-3</b> compared to that of the control.....	6
<b>Table S5.</b> PPAR-γ agonistic potency of <b>1-3</b> compared to that of the control.....	6
<b>Table S6.</b> Inhibition of NF-κB transcriptional activity compared to that of the control.....	7
<b>Table S7.</b> Inhibition of NF-κB transcriptional activity by the Perilla extract and <b>1-3</b> .....	7

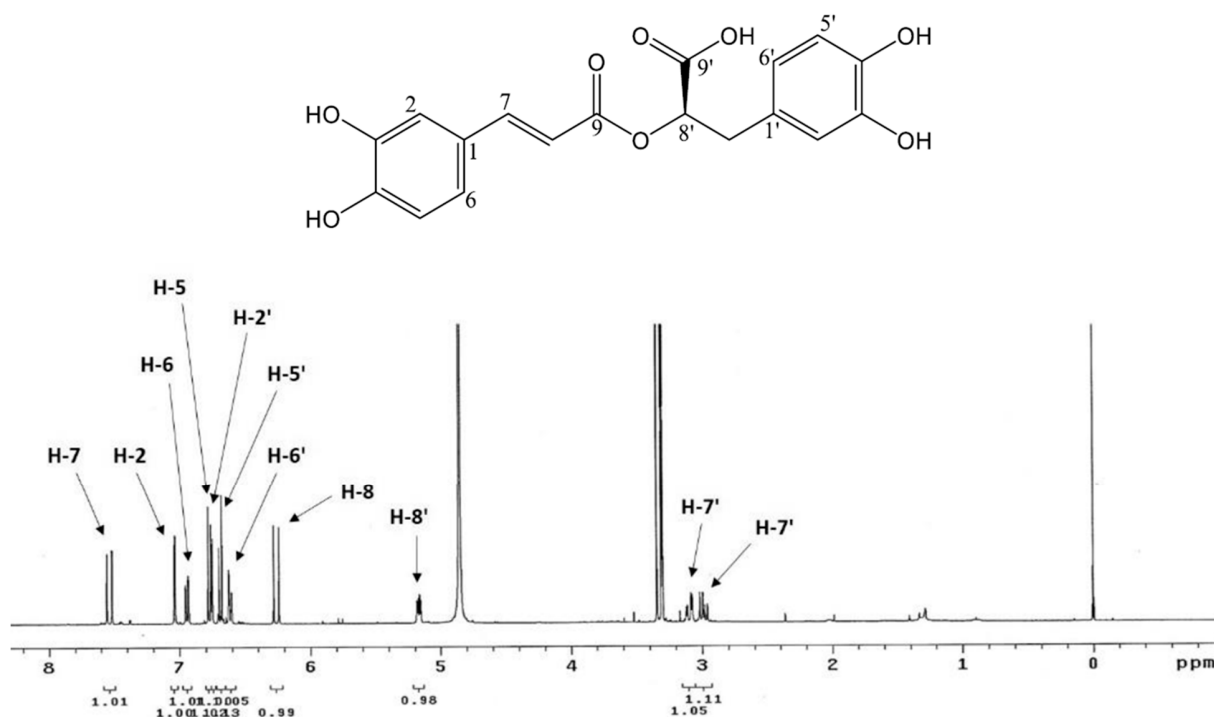




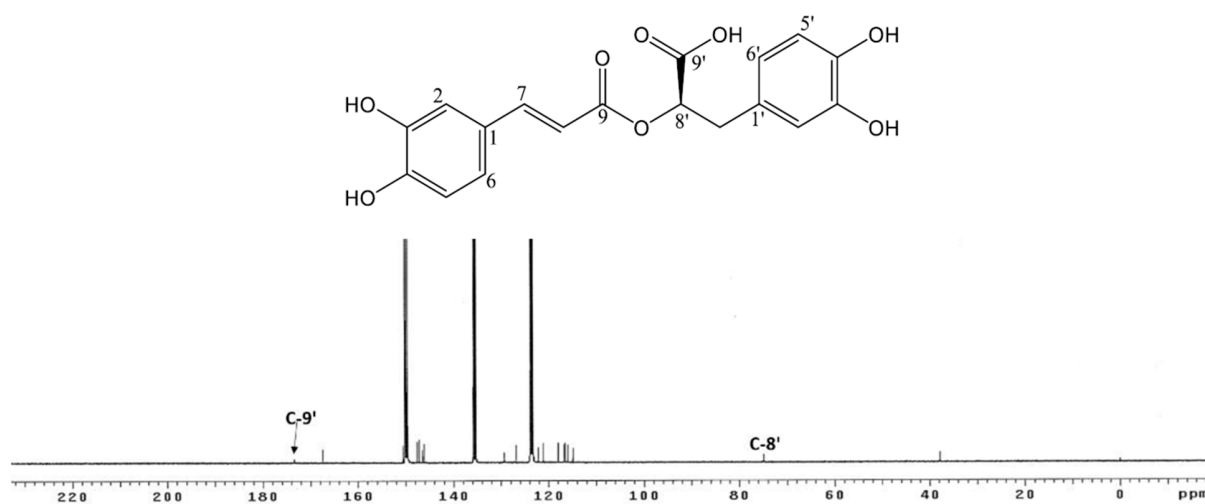
**Figure S3.**  $^1\text{H}$  NMR spectrum of compound 2 ( $\text{pyridine-}d_5$  as solvent)



**Figure S4.**  $^{13}\text{C}$  NMR spectrum of compound 2 ( $\text{pyridine-}d_5$  as solvent)



**Figure S5.**  $^1\text{H}$  NMR spectrum of compound **3** (methanol- $d_4$  as solvent)



**Figure S6.**  $^{13}\text{C}$  NMR spectrum of compound **3** (methanol- $d_4$  as solvent)

**Table S1.** Primer sequences used for quantitative real-time PCR.

Gene	Forward Primer	Reverse Primer
<i>18S</i>	ATC CCT GAG AAG TTC CAG CA	CCT CTT GGT GAG GTC GAT GT
<i>Il6</i>	TTC CTC TCT GCA AGA GAC TTC C	TGA AGT CTC CTC TCC GGA CTT
<i>Mcp1</i>	CAC TCA CCT GCT GCT ACT CA	GCT TGG TGA CAA AAA CTA CAG C
<i>Tnfa</i>	AGC CCC CAG TCT GTA TCC TT	CTC CCT TTG CAG AAC TCA GG

**Table S2.** The cell viability (%) of Raw 264.7 cells using an MTT assay.

Extract			1			2		3	
Conc. (µg/mL)	CV <sup>a</sup> (%)	Stdv <sup>b</sup>	Conc. (µM)	CV <sup>a</sup> (%)	Stdv <sup>b</sup>	CV <sup>a</sup> (%)	Stdv <sup>b</sup>	CV <sup>a</sup> (%)	Stdv <sup>b</sup>
0	100.0	4.0	0	100.0	0.5	100.0	4.2	100.0	8.5
10	97.5	2.0	5	111.4	2.9	94.7	2.2	105.7	1.4
50	80.3	2.7	10	114.6	3.3	98.7	1.1	100.8	2.5
100	81.8	0.8	50	111.0	12.8	94.3	1.9	100.6	3.0

<sup>a</sup>Cell viability. <sup>b</sup>Standard deviation.**Table S3.** PPAR-α agonistic potency of **1-3** compared to that of the control.

Blank	PPAR-α + PPRE <sup>a</sup>	WY14643	Extract <sup>b</sup>	1 <sup>c</sup>	2 <sup>c</sup>	3 <sup>c</sup>
Percentage (%)	100.00	158.36	170.93	133.30	121.61	160.58
ratio	1.00	1.58	1.71	1.33	1.22	1.61

<sup>a</sup>A Control. <sup>b</sup>A concentration of the extract: 20 µg/mL. <sup>c</sup>A concentration of **1-3**: 10µM.**Table S4.** PPAR-δ agonistic potency of **1-3** compared to that of the control.

Blank	PPAR-δ + PPRE <sup>a</sup>	GW501516	Extract <sup>b</sup>	1 <sup>c</sup>	2 <sup>c</sup>	3 <sup>c</sup>
Percentage (%)	100.00	143.36	118.84	91.23	80.34	101.92
Ratio	1.00	1.43	1.19	0.91	0.80	1.02

<sup>a</sup>A control. <sup>b</sup>A concentration of the extract: 20 µg/mL. <sup>c</sup>A concentration of **1-3**: 10µM.**Table S5.** PPAR-γ agonistic potency of **1-3** compared to that of the control.

Blank	PPAR-γ + PPRE <sup>a</sup>	Rosiglitazone	Extract <sup>b</sup>	1 <sup>c</sup>	2 <sup>c</sup>	3 <sup>c</sup>
Percentage (%)	100.00	153.79	106.44	101.85	117.22	88.58
Ratio	1.00	1.54	1.06	1.02	1.17	0.89

<sup>a</sup>A control. <sup>b</sup>A concentration of the extract: 20 µg/mL. <sup>c</sup>A concentration of **1-3**: 10µM.

**Table S6.** Inhibition of NF- $\kappa$ B transcriptional activity compared to that of the control.

	<b>1<sup>b</sup></b>				<b>2<sup>b</sup></b>			<b>3<sup>b</sup></b>		
	LPS <sup>a</sup>	5 $\mu$ M	10 $\mu$ M	50 $\mu$ M	5 $\mu$ M	10 $\mu$ M	50 $\mu$ M	5 $\mu$ M	10 $\mu$ M	50 $\mu$ M
Percentage (%)	100.00	43.17	25.04	20.14	56.66	36.42	28.86	44.14	38.79	24.72
Inhibition (%)	0.00	56.83	74.96	79.86	43.34	63.58	71.14	55.86	61.21	75.28

<sup>a</sup>A control (1  $\mu$ g/mL). <sup>b</sup>Three concentrations of **1-3** were 5, 10, and 50  $\mu$ M.

**Table S7.** Inhibition of NF- $\kappa$ B transcriptional activity by the Perilla extract and **1-3**.

<b>Extract</b>				<b>1</b>			
Conc.	<i>Il6/18s</i>	<i>Mcp1/18s</i>	<i>Tnfa/18s</i>	Conc.	<i>Il6/18s</i>	<i>Mcp1/18s</i>	<i>Tnfa/18s</i>
<b>0 <math>\mu</math>g/mL</b>	0.00	0.00	0.00	0 $\mu$ M	0.00	0.00	0.00
10 $\mu$ g/mL	-17.74	45.96	37.57	5 $\mu$ M	-38.76	-39.62	3.74
50 $\mu$ g/mL	0.69	56.95	61.44	10 $\mu$ M	-28.72	-44.71	4.32
100 $\mu$ g/mL	24.14	63.27	77.24	50 $\mu$ M	79.00	67.94	49.09
<b>2</b>				<b>3</b>			
Conc.	<i>Il6/18s</i>	<i>Mcp1/18s</i>	<i>Tnfa/18s</i>	Conc.	<i>Il6/18s</i>	<i>Mcp1/18s</i>	<i>Tnfa/18s</i>
0 $\mu$ M	0.00	0.00	0.00	0 $\mu$ M	0.00	0.00	0.00
5 $\mu$ M	-36.21	-69.95	6.09	5 $\mu$ M	7.20	30.69	27.74
10 $\mu$ M	-30.89	-44.39	19.34	10 $\mu$ M	28.56	53.88	39.56
50 $\mu$ M	80.97	44.65	21.98	50 $\mu$ M	31.50	35.74	31.81