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

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**A graph of a chemical structure

Description automatically generated**

**Figure S1.** 1H NMR spectrum of compound **1** (pyridine-*d*5 as solvent)

A diagram of a chemical structure

Description automatically generated

**Figure S2.** 13C NMR spectrum of compound **1** (pyridine-*d*5 as solvent)

A diagram of a chemical structure

Description automatically generated

**Figure S3.** 1H NMR spectrum of compound **2** (pyridine-*d*5 as solvent)

A diagram of a chemical structure

Description automatically generated

**Figure S4.** 13C NMR spectrum of compound **2** (pyridine-*d*5 as solvent)

A diagram of a chemical structure

Description automatically generated

**Figure S5.** 1H NMR spectrum of compound **3** (methanol-*d*4 as solvent)

A diagram of a chemical structure

Description automatically generated

**Figure S6.** 13C 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 |
| *18S* | ATC CCT GAG AAG TTC CAG CA | CCT CTT GGT GAG GTC GAT GT |
| *Il6* | TTC CTC TCT GCA AGA GAC TTC C | TGA AGT CTC CTC TCC GGA CTT |
| *Mcp1* | CAC TCA CCT GCT GCT ACT CA | GCT TGG TGA CAA AAA CTA CAG C |
| *Tnfa* | 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) | CVa (%) | Stdvb | Conc. (µM) | CVa (%) | Stdvb | CVa (%) | Stdvb | CVa (%) | Stdvb |
| 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 |

aCell viability. bStandard deviation.

**Table S3.** PPAR-α agonistic potency of **1**-**3** compared to that of the control.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Blank | PPAR-α + PPREa | WY14643 | Extractb | 1c | 2c | 3c |
| 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 |

aA Control. bA concentration of the extract: 20 µg/mL. bA concentration of **1**-**3**: 10µM.

**Table S4.** PPAR-δ agonistic potency of **1**-**3** compared to that of the control.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Blank | PPAR-δ + PPREa | GW501516 | Extractb | 1c | 2c | 3c |
| 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 |

aA control. bA concentration of the extract: 20 µg/mL. bA concentration of **1**-**3**: 10µM.

**Table S5.** PPAR-γ agonistic potency of **1**-**3** compared to that of the control.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Blank | PPAR-γ + PPREa | Rosiglitazone | Extractb | 1c | 2c | 3c |
| 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 |

aA control. bA concentration of the extract: 20 µg/mL. bA concentration of **1**-**3**: 10µM.

**Table S6.** Inhibition of NF-κB transcriptional activity compared to that of the control.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 1b | | | 2b | | | 3b | | |
|  | LPSa | 5 µM | 10 µM | 50 µM | 5 µM | 10 µM | 50 µM | 5 µM | 10 µM | 50 µM |
| 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 |

aA control (1 µg/mL). bThree concentrations of **1**-**3** were 5, 10, and 50 µM.

**Table S7.** Inhibition of NF-κB transcriptional activity by the Perilla extract and **1**-**3**.

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