Supporting Information to:

Hinokiresinol Inhibits IgE-induced Mouse Passive Cutaneous Anaphylaxis Reaction

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Materials and Methods

**Isolation of hinokiresinol**

The dried whole plant of *T. pseudoincisa* S. at Z. (1.1 kg) was extracted with 80% aqueous methanol (MeOH, 5 L × 2), and evaporated under vacuum. The MeOH extract (82 g) was partitioned with water (1 L) and EtOAc (1 L × 2). The EtOAc fraction (TPE, 30 g) was subjected to silica gel (300 g; Merck; Whitehouse Station, NJ, USA) column chromatography (6 × 12 cm) eluted with *n*-hexane-EtOAc [10:1→5:1→3:1, v/v, 1500 mL each] (TPE-1 to TPE-23). TPE-13 [2.1 g, Ve/Vt (elution volume/total volume) 0.80 – 0.90 (*n*-hexane-EtOAc = 5:1)] was purified by ODS (200 g) column chromatography (3.5 × 10 cm) eluted with MeOH-water (1:1→3:1, v/v, 1000 mL each) (TPE-13-1 to TPE-13-24). TPE-13-9 [125 mg, Ve/Vt 0.55 – 0.68 (MeOH-water = 1:1), Rf value on silica gel TLC in CHCl3-MeOH (10:1) = 0.5] was confirmed to be a pure compound which was identified by comparison with previous literature reports [1], [2]. The purity of the compound was evaluated as higher than 97% based on TLC [silica gel TLC in CHCl3-MeOH (10:1); ODS TLC in MeOH-water (1:1)] and 1H- and 13C-NMR spectra of the compound.

*PTE-13-9 (hinokeresinol, 125 mg)*; brown oil; [α]D20 +112° (c 0.4, MeOH); IR (KRr): ν = 3600, 3400 (br), 1610, 1250 cm⁻¹; HR-FAB-MS *m/z* = 252.1151 [M⁺].

**Sulforhodamine B (SRB) cytotoxicity assay**

Cytotoxic activity of hinokiresinol against RBL-2H3 cells was investigated using the SRB assay as described previously [3]. RBL-2H3 cells (2 × 10⁴ cells/well) grown in DMEM (Sigma; St Louis, MO, USA) supplemented with 10% fetal bovine serum and L-glutamine were seeded into 96-well plate and incubated at 37 °C with 5% CO₂ for 24 h. Various concentrations of hinokiresinol were added to each well in triplicate, then incubated at 37 °C with 5% CO₂ for 6 h. After incubation, 50 mL of cold 50% trichloroacetic acid solution were gently added to the wells. Microplates were left for 1 h at 4 °C, washed five times with tap water. Then, 0.1 mL of 0.4% SRB (Sigma; St Louis, MO, USA) solution were added to each well and left at room temperature for 30 min. SRB was removed, and the plates were washed five times with 1% acetic acid before air drying. Bound SRB was solubilized with 0.15 mL of 10 mM unbuffered Tris-base solution and plates were left on a plate shaker for at least 10 min. The optical density was measured using a microplate reader with a 540nm wavelength.
Hinokiresinol at a dose of 200 µM did not show cytotoxicity against RBL-2H3 cells (Fig. 1S).

Fig. 1S Cytotoxicity of hinokiresinol against RBL-2H3 cells.

References