Supporting Information to:

**Anti-HIV Activities of the Compounds Isolated from *Polygonum cuspidatum* and *Polygonum multiflorum***

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Materials and methods

Cytotoxicity assay: Cells were seeded in the absence or presence of various concentrations (200 μg/mL, 40 μg/mL, 8 μg/mL, 1.6 μg/mL, or 0.32 μg/mL) of extracts or compounds in triplicate and incubated at 37 °C in a humidified atmosphere of 5% CO₂ for 72 h. The supernatants were discarded and MTT reagent (5 mg/mL in PBS) was added to each well and then incubated for 4 h, after which 100 μL of 50% DMF–20% SDS was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek Elx 800 ELISA reader at 595/630 nm. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC₅₀) was calculated from the dose–response curves.

Syncytium reduction assay: In the presence of 100 μL of various concentrations of extracts and compounds, C8166 cells (8 × 10⁵ /mL) were seeded and incubated with 1300 TCID₃₀ HIV-1₃Ⅰ and then incubated at 37 °C in a humidified incubator with 5% CO₂ for 72 h. Control assays were performed without the testing compounds in HIV-1-infected and -uninfected cultures. AZT was used as positive control. After 3 days of co-culture, the cytopathic effect was measured by counting the number of syncytia (multinucleated giant cells) in each well under an inverted microscope. Five concentrations, 200 μg/mL, 40 μg/mL, 8 μg/mL, 1.6 μg/mL, and 0.32 μg/mL, of each compound or extract were used to generate dose–response curves. The percentage inhibition of syncytial cell formation was estimated from the percentage of syncytial cell number in treated culture to that in infected control culture, and the 50% effective concentration (EC₅₀) was calculated. The therapeutic index (TI) = CC₅₀ / EC₅₀.

Statistical analysis

Statistical calculations were carried out with SPSS 10.0 software. The results are expressed as the mean ± SD of three independent experiments. Student’s *t*-test was used for statistical analyses; *P* values < 0.05 were considered to be significant.