



Additional Figure 1 Direct effect of CCAD on AchE activity.

AchE activity was measured by the colorimetric assay. (A) 5 μL AchE (1 unit/mL) and 5 μL H₂O₂ at different concentrations (400, 200, 100, 50, 25, and 0 μM) were mixed and incubated for 10 minutes before the reaction. The solutions were then reacted with Ellman’s reagent and the substrate. (B) 5 μL AchE (1 unit/mL) and 5 μL samples (NC, DDW; donepezil, 10 μM; CCAD, 0.5 mg/mL; FGD, 0.5 mg/mL) were mixed and incubated for 10 minutes before the reaction. (C) 5 μL AchE (1 unit/mL, and the final concentration is 0.5 unit/mL), 2.5 μL samples (donepezil, 10 μM; CCAD, 0.5 mg/mL; FGD, 0.5 mg/mL), 2.5 μL H₂O₂ at 400, 200 and 100 μM were mixed and incubated for 10 minutes before the reaction. As the positive control, 2.5 μL DDW was mixed with 2.5 μL H₂O₂ at differentiations. AchE: Acetylcholinesterase; CCAD: enzyme-digested *Colla Corii Asini*; DDW: double distilled water; FGD: enzyme- digested fish gelatin; NC: negative control.