

Cytotoxic Effects of *Citrus aurantifolia* Crude Leaf Extracts on Vero Cells and C6/36 Cells Prior to Test the Anti-Dengue Viral Activity of the Extracts

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Citrus aurantifolia is a traditionally used medicinal plant, which can be tested against Dengue-virus (DENV) infections. The objective of this study was to identify *in vitro* cytotoxicity of *C. aurantifolia* leaf extracts against normal African green monkey kidney epithelial (Vero) cells and *Aedes albopictus* (C6/36) cells prior to antiviral studies against DENV infections. CytoTox-96®-Promega-USA is a colorimetric assay that quantitatively measures lactate dehydrogenase released upon cell lysis. *C. aurantifolia* leaves were ground and fresh, neat extract was diluted in normal saline to prepare the two-fold dilution series. Two 96-well assay plates were prepared separately with Vero and C6/36 cells. The assay was set up using CytoTox-96®-Promega-USA-kit manufacturer's instructions, including 1. Negative control-without cells, 2. Vehicle control-untreated cells, 3. Positive control-lysis solution with four replicates. Leaf extract was added into the test wells at different concentrations and plates were incubated for 5 and 24 hours at 37 °C and at 28 °C. The absorbance data were measured using a standard 96-well plate reader (Labtech-LT-4500-Singapore) and the percentage cytotoxicity was calculated. The cytotoxicity-driven colour intensity, absorbance values and percentage cytotoxicity decreased with the decreasing concentrations of leaf extract. The percentage cytotoxicity against Vero cells for dilutions of 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024 were 104.99, 83.96, 75.33, 82.96, 22.23, 35.56, 25.97, 11.26, 5.82, respectively, for the 5-hour incubation and 104.55, 91.16, 53.26, 45.83, -17.61, -31.52, -34.47, -30.51, -25.73, respectively for the 24-hour incubation. The percentage cytotoxicity against C6/36 cells were 99.42, 80.34, 50.09, 36.46, 12.23, 7.12, 7.44, -7.19, -8.99, respectively for the 5 hours and 119.10, 96.59, 63.43, 42.04, 31.42, 20.14, 9.21, 5.19, 1.73, respectively for the 24 hours. High leaf extract concentrations were cytotoxic to both Vero and C6/36 cells. Cytotoxicity of *C. aurantifolia* leaf extracts helps select the minimum toxic concentrations for testing the anti-viral activity against DENV infections.

Keywords: Absorbance, *Citrus aurantifolia* leaf extract, Colour intensity, Cytotoxicity

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