QUANTITATIVE APPROACH IN SCREENING THE ANTIVIRAL PROPERTIES OF KANDIS HUTAN IN ANIMAL CELL CULTURE

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Abstract

This main aim of this study was to determine the concentration of Kandis Hutan leaf extracts that can inhibit the infectivity of pseudorabies virus (PrV) in Vero cells. The leaf extracts were crude and extracted with 3 organic solvents namely hexane, ethyl acetate and ethanol. The cytotoxic effect of extracts on Vero cells was assessed by both MTT assay and cell cytotoxicity scoring method. Two-fold serial dilutions of each extracts were prepared from the highest concentration of 1000µg/ml in 0.1% DMSO. For MTT assay, the highest cytotoxicity was found in the hexane extract (CC_{50} <1.25 µg/mL), followed by ethyl acetate extract (CC_{50} = 237.5 µg/mL), whilst minimal cell cytotoxicity was observed in ethanol extracts (CC_{50} = 555.0 µg/mL). There was a significant correlation between cell scoring system and MTT assay in term of cell cytotoxicity whereby the least toxic was ethanol extracts, followed by ethyl acetate extract and the most toxic hexane extract. Four antiviral assays were conducted for each extracts, namely plaque reduction assay, cytopathic effect (CPE) reduction assay, inhibition assay and virucidal assay. The most promising result was obtained from the inhibition assay, in which ethyl acetate extracts produced 75% viral inhibition at 125 µg/mL concentration. In virucidal assay, both ethyl acetate and ethanol extracts produced 100% viral inhibition at 250 µg/mL. For plaque reduction assay, there was a significant dose dependent inhibition for ethyl acetate extract but not for ethanol extract and hexane extract. In comparison to CPE reduction assay, findings from plaque reduction assay showed better viral inhibition by ethyl acetate extract (47%) at concentration of 300 µg/mL. The estimated selectivity index (ESI) calculated from the inhibition assay showed the highest antiviral response by ethyl acetate extract (2.7) in comparison with ethanol extract (1.8) and hexane extract (0.1). Therefore, the most promising antiviral activity was produced by ethyl acetate extract which showed consistent viral inhibition in all tested antiviral assays. In contrary, hexane extracts showed the least antiviral efficacy among the tested extracts.

Keywords: pseudorabies virus (PrV), Kandis Hutan, antiviral assay, plaque reduction assay, inhibition assay, virucidal assay