

7th Proceedings of the Seminar on Veterinary Sciences, 27 February – 02 March 2012

QUANTITATIVE *IN VITRO* ASSAYS FOR SCREENING ANTIVIRAL CHARACTERISTICS OF BEREMBANG BUKIT LEAF EXTRACTS

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Abstract

Berembang Bukit or Megawasih, is a native of our tropical rainforest and other ASEAN countries. In a preliminary review, a qualitative screening on the antiviral activities of its leaf extracts revealed various degrees of antiviral potential. Therefore this study was done to evaluate its antiviral characteristics in a quantitative approach. The leaf extracts were prepared using hexane, ethyl acetate and ethanol extractions and dissolved in DMSO. DMSO cytotoxicity was initially evaluated to ensure a safe working concentration. A negligible cytotoxicity was observed at a concentration of $\leq 0.1\%$ DMSO. 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 $1000\mu\text{g/mL}$ in 0.1% DMSO. For MTT assay, the highest cytotoxicity was found in the ethyl acetate extract ($CC_{50} = 218\mu\text{g/mL}$), whilst minimal cell cytotoxicity was observed in both hexane ($CC_{50} = 833\mu\text{g/mL}$) and ethanol ($CC_{50} = >1000\mu\text{g/mL}$) extracts. However, there were no correlation between MTT and cell scoring for cytotoxicity in this study. A series of experiments including CPE reduction assay, plaque reduction assay, inhibition assay and virucidal assay were done to evaluate the total antiviral potential of the leaf extracts. The leaf extracts produced a dose-dependent antiviral response. Both ethyl acetate and ethanol extracts showed 100% plaque formation inhibition in plaque reduction assay, inhibition assay and virucidal assay. Hexane extracts showed absence of plaque inhibition in all the tested antiviral assays. In inhibition assay, the estimated selective index (ESI) for ethanol and ethyl acetate extracts were 8.3 and 1.9, respectively. Whilst in CPE reduction assay, the ESI for the respective extracts were 6.7 and 2.9. In conclusion, the ethanol extracts exhibited the highest antiviral efficacy among the tested extracts.

Keywords: antiviral assay, Berembang Bukit, plaque reduction assay, inhibition assay, virucidal assay