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RESEARCH NOTES

CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF *Melastoma malabathricum* EXTRACTS

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Melastoma malabathricum L. is widely used in Malay traditional practices utilizing each and every part of this plant (Burkill, 1966). It is scientifically proven that this plant has several properties including antibacterial (Zulaikah *et al.,* 2008), antiviral (Lohezic-Le Devehat *et al.,* 2002) antioxidant (Susanti *et al.,* 2007) and anti inflammatory (Mazura *et al.,* 2007) activities. Despite the successes in the treatment of some virus diseases during the past three decades, the search for new antiviral drugs remains an area of active investigation. Effective treatment is not available for many viral infections. Moreover, the selection of resistant and cross-resistant mutants caused partially by the narrow spectrum of the mechanism of activity, as well as potential toxic side-effects demand the discovery of new drugs.

Measles virus is an enveloped virus, single-stranded RNA virus in the family *Paramyxoviridae.* Measles remains a leading cause of death among young children, despite the availability of a safe and effective vaccine for the past 40 years. World Health Organization (WHO) estimated 242 000 people, the majority of them children, died from measles in 2006 (WHO, 2007). Herpes simplex virus type 1 (HSV-1) is an enveloped double-stranded DNA virus of the *Herpesviridae* with the ability to establish life long latency after primary infection. In this study, we investigated the cytotoxicity of *M. malabathricum* methanol extract (MMME) against cells lines and its potential in protecting cells against viral infection.

Air-dried *M. malabathricum* leaves were extracted with methanol and left to dry by rotary evaporator (Heidolph) to produce crude *M. malabathricum* methanol extract (MMME). Thin layer chromatography was performed to separate components in MMME on Silica gel (F250, Sigma) as solid phase and developed in chloroform and methanol (4 to 1). Rutin, quercetin and quercitrin served as standards.

In cytotoxicity screen, Vero cell line (African green monkey, *Cercopitheus aethiops* kidney cells) and L929 cells (mouse fibroblast) were grown in Dulbecco's Minimal Essential Medium (DMEM) enriched with 10% fetal bovine serum (FBS) (Flowlab, Australia), 100mg/ml of streptomycin and 100 IU/ml of penicillin in a humidified atmosphere of 5% CO_2 at 37ºC. Cytotoxicity test was done in at least two independent experiments in triplicates at different concentrations of MMME using doubling dilutions from initial stock concentration of 1000µg/ml. The cytotoxicity test was performed on confluent cells according to the microculture method (Marini *et al.*, 1998) and cells were stained with Eosin B after 48 h of incubation. Readout from each well was normalized against the absorbance from empty wells and LC_{50} was presented as the percentage of survived cells compared to control cells according to the formula by Schmidtke *et al.* (2001). The LC_{50} value for guanidine hydrochloride (GHCl) that served as positive control in the antiviral study was also determined.

As for antiviral screen, HSV-1 and vaccine strain of measles (Schwarz) titres were calculated by the above method and $TCID_{50}$ values were estimated. Three methods of treatments to detect antiviral activity in each of the fraction were used: (i) cells (C) were inoculated with virus (V) 1 hour before treatment with extract (E), that is (C+V) + E; (ii) virus was inoculated to cells one day after treatment with extract, that is (C+E) + V; and (iii) the virus and extract were added concurrently to the cells, that is C + (V+E) (Nurul Aini *et al.,* 2006). For the antiviral tests, the extract was diluted at 1.0 LC_{50}, 0.1 LC_{50} and 0.01LC_{50}. The viral concentrations used for cell inoculations were fixed at 1 $TCID_{50}$. Percentage of survived cells

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after being treated is compared to control cells as above and degree of antiviral activity was determined in accordance to this percentage.

Separation of MMME by TLC revealed three components with Rf values of 0.05, 0.26 and 0.69 that corresponds to three standard components namely rutin, quercitrin and quercetin. These flavonoid components have been proven to display antimicrobial (Lohezic-Le Devehat *et al.,* 2002, Zulaikah *et al.,* 2008) and anti inflamatory (Mazura *et al.,* 2007) properties.

Cytotoxicity screen towards Vero and L929 cells showed that *M. malabathricum* methanol extract was not cytotoxic to both cells with LC_{50} values of 750 µg/ml and >1000 µg/ml respectively. As for GHCl, it was found not cytotoxic in Vero cells with LC_{50} value of 100µg/ml. LC_{50} value was not determined for L929 cells.

In this study, *M. malabathricum* methanol extract showed antiviral activity with different modes of action against HSV-1 or measles viruses (Table 1). MMME was effective in inhibiting cell death by 0.01LC_{50} HSV-1 inoculated cells using treatment mode [(C+V)+E]. Positive effect to this mode suggests that virus inoculated cells were able to overcome viral infection when treated with MMME. The result from this study confirms the results obtained by Lohezic-Le Devehat et al. (2002) that *M. malabathricum* extract has moderate effect on HSV-1. The possible mode of action is the removal of HSV-1 infectivity by MMME during early stages of viral replication particularly the attachment stage. In HSV-1 infection, adsorption and penetration was achieved by 2 hours post-infection (Honess and Roizman, 1974) with immediate-early proteins of HSV-1 are synthesized 1-2 hours post-infection (Dixon and Schaffer, 1980).

Cells treated with simultaneous addition of measles virus and MMME at 0.1 and 1 LC_{50} were found to survive from viral infection (Table 1). The effect of MMME is probably due to the quercetin content that can inhibit reverse transcriptase (Spedding et al. 1989) which is the early part of the measle's replication process. Another possibility is that MMME is capable to act directly on viral particle such as modification of free viruses surfaces' that inhibits viral attachment to host cells. This belief was supported by the fact that MMME was not capable of inhibiting virus inoculated cells in treatment mode I. However, the most probable mode of infection can only be further confirmed by transcriptomic or proteomic studies.

MMME was found not to have prophylactic effect on both test viruses as demonstrated in treatment mode (C+E) + V. As for control, GHCl is seen in this study to inhibit viral capability to infect host cells when added simultaneously. GHCl is known to block the initiation step of viral RNA synthesis (Baltimore *et al.* 1963).

Table 1. Antivirus activity of *M. malabathricum* methanol extract in Vero cells inoculated with HSV-1 and measles viruses. The activity is presented as the percentage of cell survival and degree of effectiveness* in brackets. Vero cells were treated using 3 different treatment modes: [(C+V)+E], [(C+E)+V] and [C+(E+V)] at 3 different concentrations. Viral inoculation dose for both viruses was set at 1 $TCID_{50}$

Treatment mode at different LC50 values	Test virus	
	HSV-1	Measles virus
[(C+V)+E]		
0.01	229% (+)	87% (–)
0.1	116% (+)	96% (±)
1	78% (–)	46% (–)
(C+V)+G	87% (–)	93% (–)
[(C+E)+V]		
0.01	91% (±)	52% (–)
0.1	75% (–)	57% (–)
1	58% (–)	46% (–)
(C+G)+V	92% (±)	83% (–)
[C+(E+V)]		
0.01	83% (–)	91% (±)
0.1	53% (–)	128% (+)
1	74% (–)	194% (+)
[C+(G+V)]	115% (+)	101% (+)

Notes: *Degree of effectiveness: – percentage of cell survival is less than 90%; ± percentage of cell survival is more than 90% but less than 100%; + percentage of cell survival is more than 100%; G=Positive control i.e. treated with GHCl instead of extract.

In conclusion, *M. malabathricum* methanol extract was found to be non-cytotoxic to kidney and fibroblast cell lines. Further, MMME was also capable in inhibiting HSV-1 and measles viruses during the early stages of viral replication.

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