Title : Inhibition of Dengue virus infection by *Clinacanthus nutans* (*C. nutans*) extract combined with Melittin pe
the ability of *C. nutans* extract to increase cell viability.

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ABSTRACT

Dengue virus (DENV) is the causative agent of dengue fever, a disease that can be transmitted from human to human through the *Aedes aegypti* mosquito, which serves as its primary vector. Upon infection, the severity of the disease can range from mild symptoms to fatal outcomes. Over the past several years, the number of dengue-related fatalities has continued to increase annually, particularly in tropical regions. This is primarily due to the absence of specific antiviral treatments or targeted therapeutic agents. Consequently, current treatment strategies focus on symptomatic management and preventive measures to minimize the risk of infection. This study aims to develop an extract with inhibitory activity against dengue virus serotype 2 (DENV-2) by utilizing the synergistic antiviral effects of two bioactive compounds: Melittin, a peptide derived from bee venom, and an extract from *Clinacanthus nutans* (commonly known as "Phaya Yo" in Thai). Both compounds have previously been reported to exhibit potent antiviral properties. The cytotoxicity of these compounds was assessed using a cell viability assay on Vero cells. The results indicated that Melittin at concentrations of 1.25–2.5 µg/mL and *C. nutans* extract at concentrations of 15.625–500 µg/mL maintained cell viability above 80%. Conversely, Melittin at a concentration of 5 µg/mL significantly reduced cell viability to below 50%. However, when Melittin at 5 µg/mL was combined with *C. nutans* extract, the extract enhanced cell survival, increasing viability to over 80%. The viral entry inhibition was evaluated using the FFU reduction assay and the Cell-based Enzyme-Linked Immunosorbent Assay (ELISA). The findings demonstrated that Melittin at a concentration of 1.25 µg/mL inhibited viral entry by more than 50%, while at concentrations of 2–5 µg/mL, viral inhibition reached 100%. Similarly, *C. nutans* extract at 7.8125 µg/mL inhibited viral entry by over 70%, and at concentrations of 15.625–250 µg/mL, it achieved 100% inhibition. Notably, the combination of Melittin and *C. nutans* extract at concentrations of 7.8125–125 µg/mL demonstrated superior viral entry inhibition compared to either compound used individually. Furthermore, the FFU titration assay was performed to assess the production of newly synthesized viral particles, while the Immunofluorescence Assay (IFA) was used to quantify the total number of infected cells. The results suggest that both extracts effectively reduce viral infection rates. When comparing the antiviral efficacy of the individual compounds to their combined use, the combination exhibited significantly greater inhibitory activity against the virus. Therefore, these naturally derived compounds represent a promising alternative for further development as potential therapeutic agents for dengue virus infection in the future.