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

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Abstract

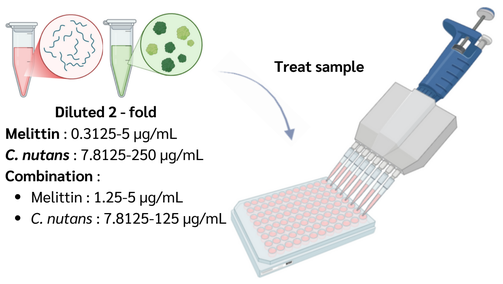
Dengue virus (DENV) is the causative agent of dengue fever, a disease that can be transmitted from human to human through the Aedes aegypti mosquito, which serves as its primary vector. Upon infection, the severity of the disease can range from mild symptoms to fatal outcomes. Over the past several years, 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, while viral protein production was evaluated using 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.

Objectives

- To investigate whether *C. nutans* extract can improve cell viability when combined with Melittin.
- To study the inhibitory effects of *C. nutans* extracts and Milittin peptide on the Dengue virus.
- To study the potential synergistic effects of *C. nutans* extracts and Milittin peptide.

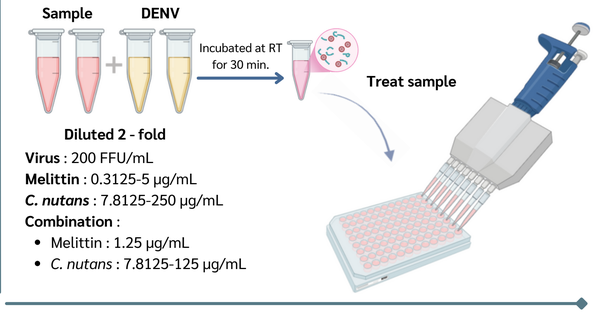
Methods

• Sample preparation for cell viability assay



Diluted 2 - fold
Melittin : 0.3125-5 µg/mL
C. nutans : 7.8125-250 µg/mL
Combination :
- Melittin : 1.25-5 µg/mL
- *C. nutans* : 7.8125-125 µg/mL

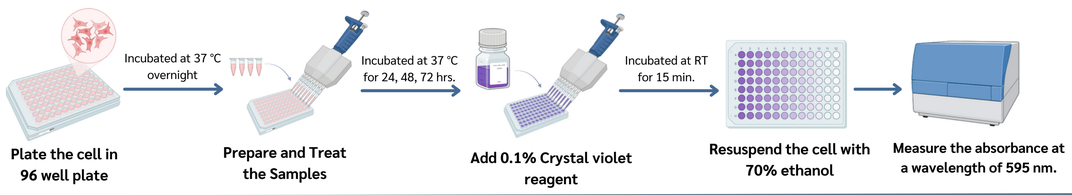
• Sample preparation for anti-viral assay



Diluted 2 - fold
Virus : 200 FFU/mL
Melittin : 0.3125-5 µg/mL
C. nutans : 7.8125-250 µg/mL
Combination :
- Melittin : 1.25 µg/mL
- *C. nutans* : 7.8125-125 µg/mL

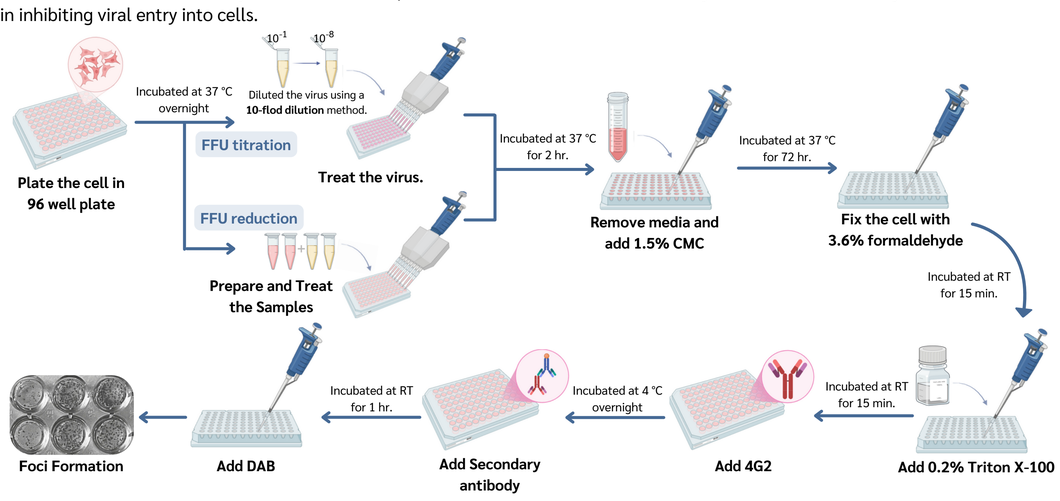
• Cell viability assay

The % cell viability assay was performed to determine the non-cytotoxic dose of the substances for antiviral testing.



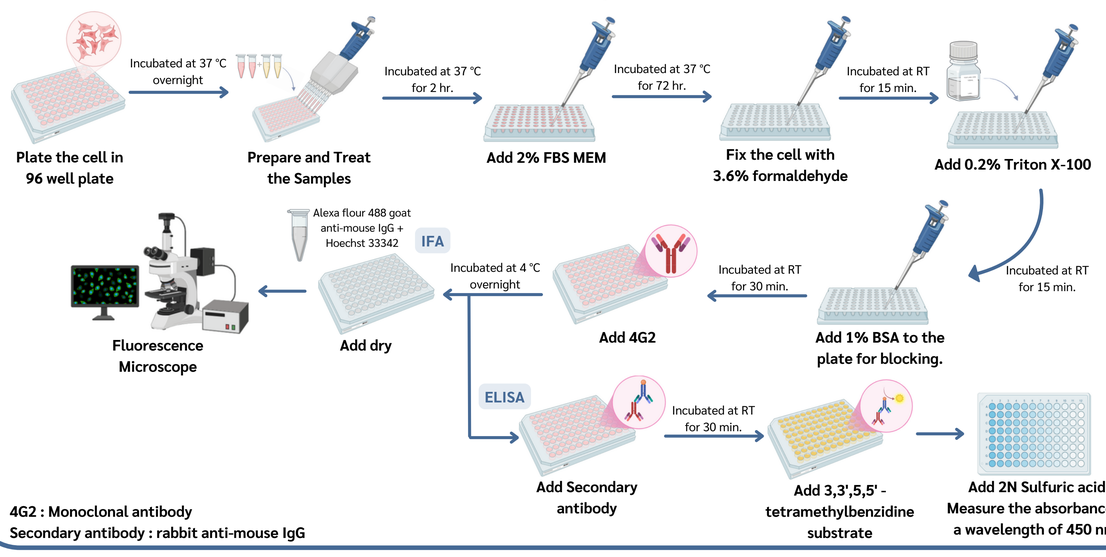
• FFU Titration assay and FFU Reduction assay

Perform the FFU titration assay to determine viral production and the FFU reduction assay to evaluate the efficacy of the substances in inhibiting viral entry into cells.



• Cell-based Enzyme-Linked Immunosorbent Assay (ELISA) and Immunofluorescence assay (IFA)

The quantification of viral protein production was performed using the ELISA method, while the total number of virus-infected cells was determined using the IFA method.



4G2 : Monoclonal antibody
Secondary antibody : rabbit anti-mouse IgG

Conclusion

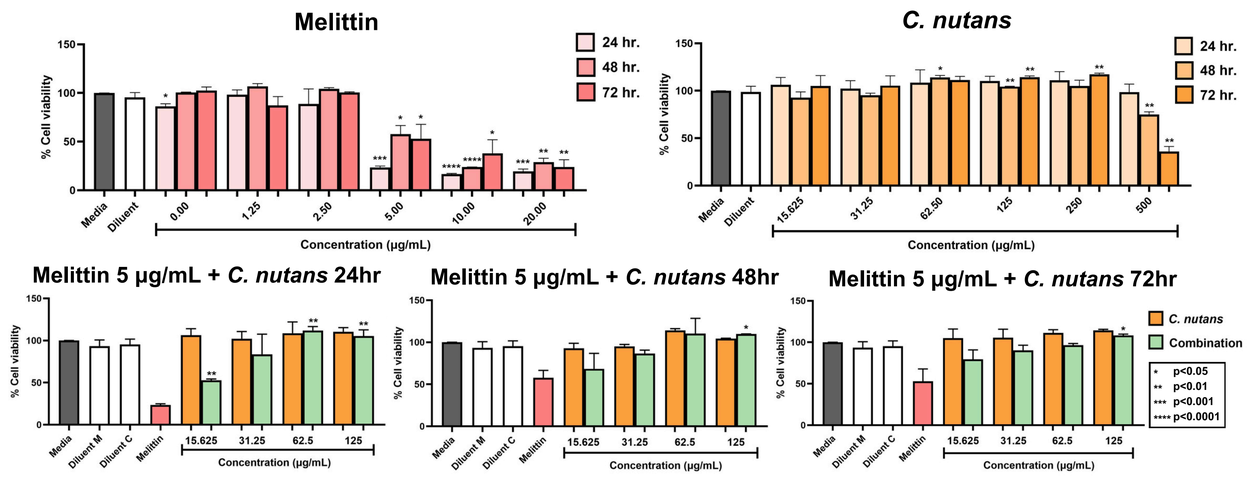
The extract of *C. nutans* was able to increase cell viability when used in combination with Melittin at a concentration of 5 µg/mL. Additionally, both substances exhibited effective antiviral activity against the dengue virus. Notably, their combined use resulted in a greater antiviral efficacy compared to the use of each compound individually, indicating a synergistic effect in viral inhibition. The researchers anticipate that these findings may serve as a potential basis for the development of dengue fever treatments in the future.

References

- Aussara Panya, Hataichanok Pundith, Supawadee Thongyim, Thida Kaewkod, Thararat Chitov, Sakunnee Bovonsombut and Yingmanee Tragoolpua. 2020. **Antibiotic-Antiapoptotic Dual Function of *Clinacanthus nutans* (Burm. f.) Lindau Leaf Extracts against Bovine Mastitis.** doi:10.3390/antibiotics9070429
- Aussara Panya, Saruda Thongyim and Terd Disayathanoowat. (n.d.). **Potential Inhibition of Dengue Virus by Melittin Peptide.** Unpublic data.
- Kanyaluck Jantakee. Suthida Panwong, Pachara Sattayawat, Sasithon Saengmuang, Kiattawee Choowongkomon, Ratchaneewan Sumankan, and Aussara Panya. 2024. ***Clinacanthus nutans* (Burm. f.) Lindau Extract Inhibits Dengue Virus Infection and Inflammation in the Huh7 Hepatoma Cell line.** doi: 10.3390/antibiotics13080705

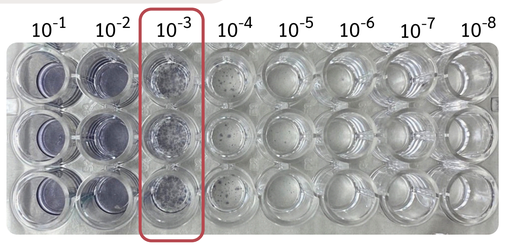
Results

Cell viability assay



- The *Clinacanthus nutans* extract can **increase %cell viability** when combined with Melittin at concentration 5 µg/mL.

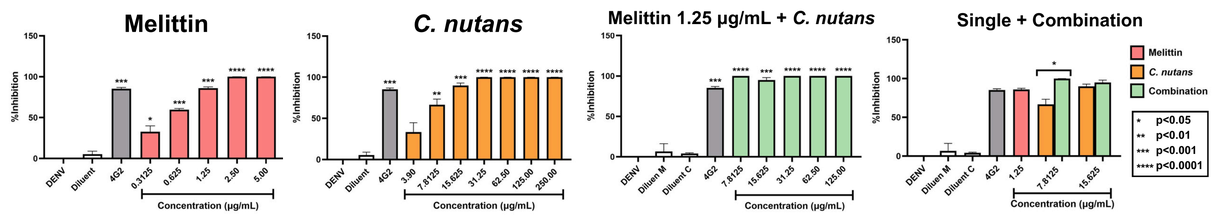
FFU Titration assay



Foci	109	112	128

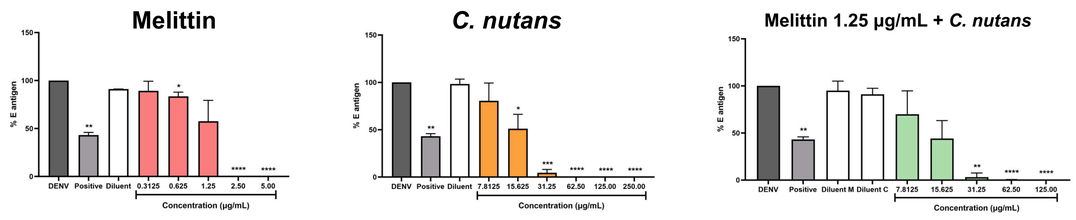
- Based on the counted number of foci, the viral titer was calculated to be **1.16 × 10⁶ FFU/mL.**

FFU Reduction assay



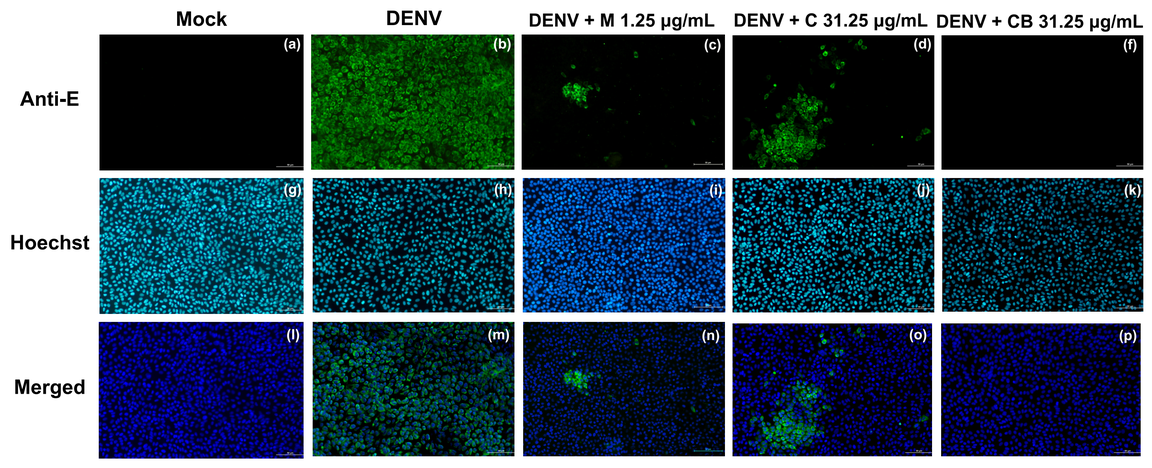
- Melittin peptide at concentrations of **2.5–5 µg/mL**, *C. nutans* extract at concentrations of **31.25–250 µg/mL**, and the **combination** of Melittin at 1.25 µg/mL with *C. nutans* extract at concentrations of 7.8125–125 µg/mL were able to **inhibit dengue virus by 100%**.
- The combination of both substances resulted in an increased % viral inhibition.

Enzyme-linked immunosorbent assay (ELISA)



- Melittin peptide at a concentration of 2.5–5 µg/mL, *C. nutans* extract at a concentration of 62.5–250 µg/mL, and the combination of Melittin at 1.25 µg/mL with *C. nutans* extract at 62.5–125 µg/mL were able to reduce %E antigen by 100%.

Immunofluorescence assay (IFA)



- Cells treated in conditions (n) and (o) exhibited a reduced viral infection compared to the control group (l). Additionally, condition (p) demonstrated a further reduction in viral infection when both compounds were used in combination.