Inhibition of herpes simplex virus type 2 by *Carissa carandas* Linn. fruits extracts

Nattida Phairat* and Yingmanee Tragoolpua

Division of Microbiology, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

E-mail : *nattida_phairat@cmu.ac.th

Abstract

Herpes simplex virus type 2 (HSV-2) is a major etiological agent of genital herpes, a sexually transmitted infection with global prevalence. Currently, antiviral drugs such as acyclovir are widely used for treatment of HSV infection. However, using these drugs have several limitations, including the emergence of drug-resistant strains, the high cost of long-term therapy, and the inability to completely eradicate latent viruses. These limitations have led to increase the interest in natural extracts as alternative therapeutic agents for the inhibition of HSV-2. This study aimed to evaluate the anti-HSV-2 activity of extracts derived from the fruits of *Carissa carandas* L. Cytotoxicities of the *C. carandas* fruit extracts were assessed on Vero cells using MTT assay. Among the tested extracts, the 95% ethanolic extract of *C. carandas* unripe fruit exhibited the lowest cytotoxicity, with a 50% cytotoxic concentration (CD_{50}) of 2.00 ± 0.25 mg/mL. Antiviral activity of *C. carandas* fruit extract against HSV-2 was evaluated using a plaque reduction assay on Vero cells. The ethanolic extract of *C. carandas* ripe fruit extract at a concentration of 0.3125 mg/mL demonstrated the highest inhibitory effect after viral attachment to host cells, with a viral inhibition rate of 96.47 ± 1.97%. Total phenolic content (TPC) analysis revealed that the ethanolic extract of *C. carandas* ripe fruit extract contained the highest phenolic level (8.00 ± 0.58 mg GAE/g extract), which was significantly higher than that of the aqueous extracts. In contrast, antioxidant activity assessed by DPPH and ABTS assays showed that the aqueous extract of *C. carandas* ripe fruit exhibited the strongest radical scavenging activity, with values of 2.12 ± 0.34 mg GAE/g extract and 29.18 ± 2.21 mg Trolox/g extract, respectively. the IC_{50} values were 6.72 ± 1.14 mg/mL and 7.98 ± 0.63 mg/mL, respectively. Therefore, *C. carandas* fruit extracts, particularly the ethanolic extract of ripe fruit, demonstrated potent inhibitory activity against HSV-2 after viral attachment to host cells. The antiviral activity appears to correlate with phenolic content and antioxidant capacity, supporting the potential development of *C. carandas* extracts as natural antiviral agent against HSV-2 in the future.

Introduction

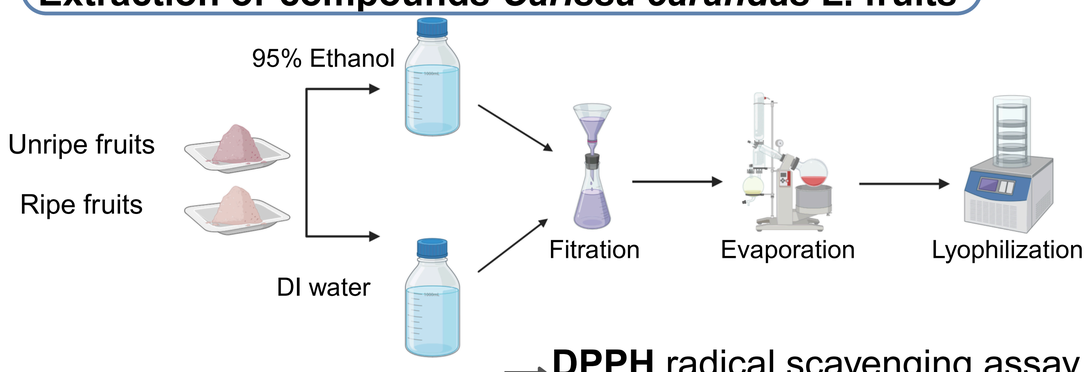
Herpes simplex virus infection is transmitted through direct contact with infectious secretions and establishes lifelong latency in sensory ganglia with potential reactivation. Herpes simplex virus type 1 (HSV-1) mainly causes oral lesions, whereas Herpes simplex virus type 2 (HSV-2) is the principal cause of recurrent genital herpes. Although antivirals such as acyclovir are widely used, they do not eliminate latent virus, and prolonged therapy may lead to drug resistance. Therefore, natural antiviral alternatives are increasingly sought. *Carissa carandas* L. is a medicinal plant that rich in phenolic and other bioactive compounds. This study aimed to evaluate the anti-HSV-2 activity of *C. carandas* fruit extracts, together with their cytotoxicity, total phenolic content, and antioxidant activity.

Objectives

1. To evaluate the cytotoxicity of crude extracts from *C. carandas* L. fruits on Vero cells.
2. To determine the efficacy of crude extracts from *C. carandas* L. fruits against HSV-2.
3. To determine the antioxidant properties of crude extracts from *C. carandas* L. fruits.

Methodology

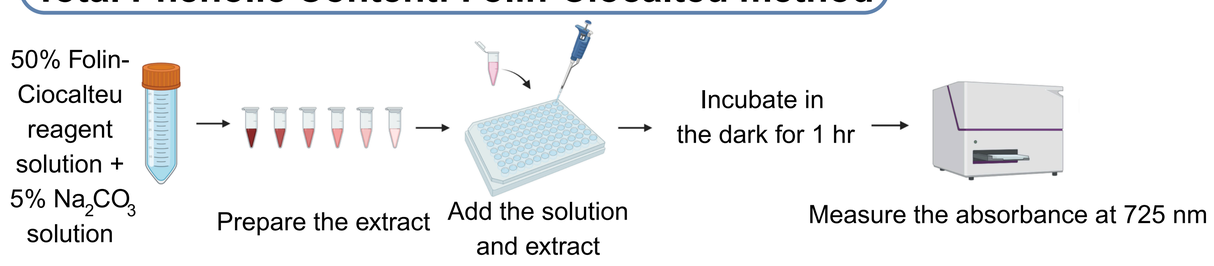
Extraction of compounds *Carissa carandas* L. fruits



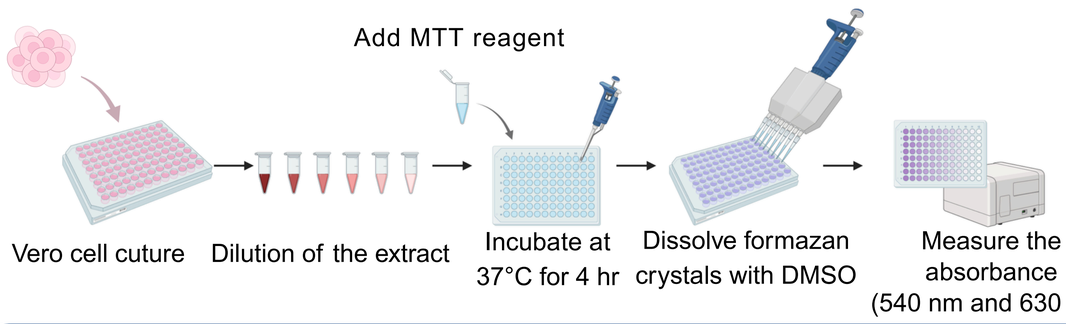
Antioxidant activity test

- **DPPH** radical scavenging assay
- **ABTS** radical scavenging assay

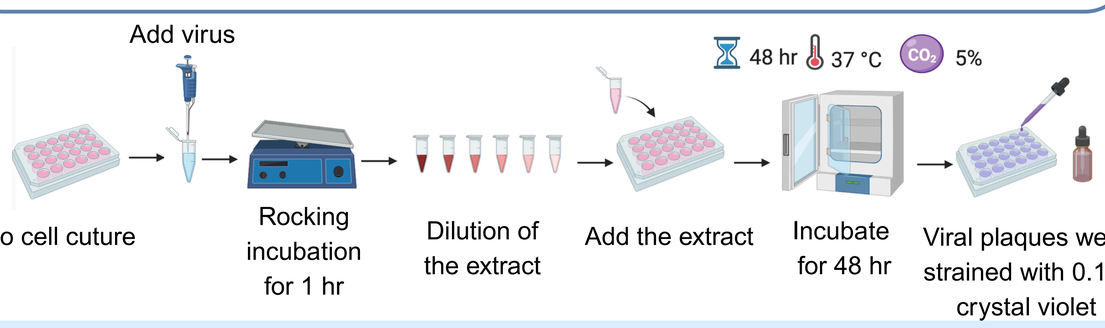
Total Phenolic Content: Folin-Ciocalteu method



Cytotoxicity test: MTT assay



Determination of the inhibitory effect of *Carissa carandas* L. extracts on HSV-2 after viral attachment by Plaque reduction assay



Results

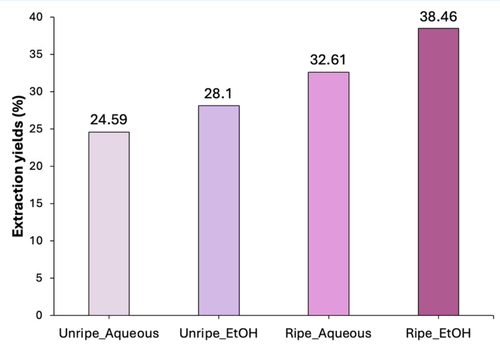


Figure 1 Extraction yield of *Carissa carandas* L. fruits extracts

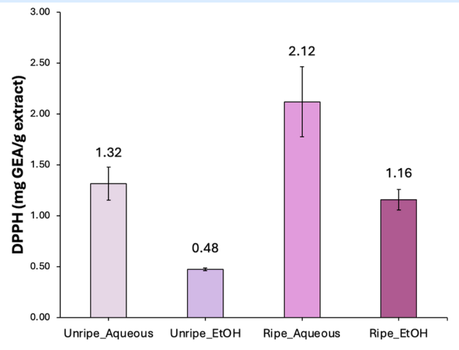


Figure 2 DPPH radical scavenging activity of aqueous and 95% ethanolic extracts of *Carissa carandas* L. fruits

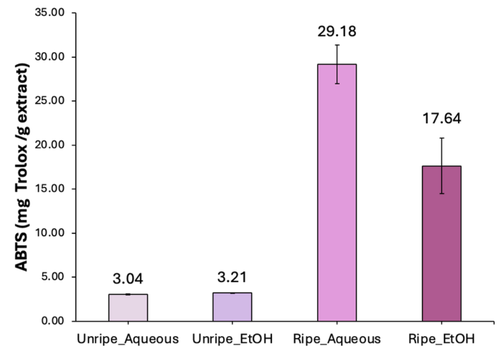


Figure 3 ABTS radical scavenging activity of aqueous and 95% ethanolic extracts of *Carissa carandas* L. fruits

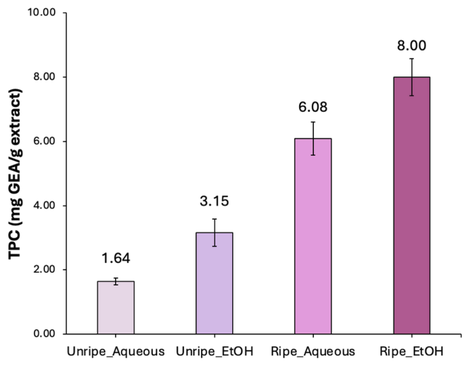


Figure 4 Total phenolic content by Folin-Ciocalteu method

Table 1 CD_{50} values of *C. carandas* L. fruits extracts determined by MTT assay

Solvent	Type of extracts	CD_{50} (mg/mL)
Aqueous	Unripe fruit	1.84 ± 0.59
	Ripe fruit	1.69 ± 0.40
Ethanol	Unripe fruit	2.00 ± 0.25
	Ripe fruit	1.69 ± 0.38

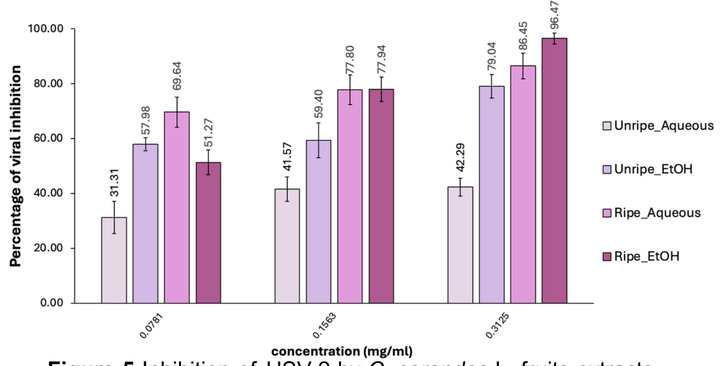


Figure 5 Inhibition of HSV-2 by *C. carandas* L. fruits extracts after viral attachment

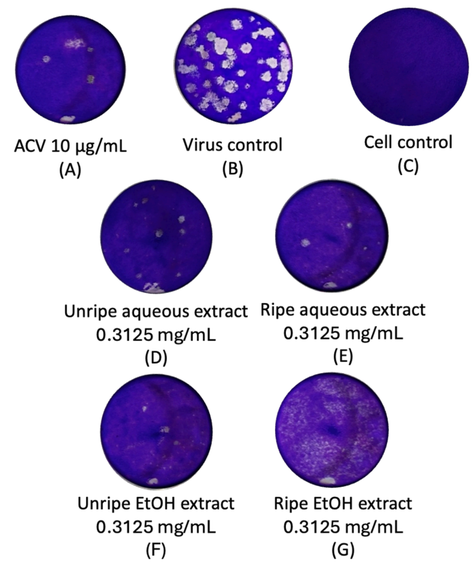


Figure 6 Plaque reduction assay of HSV-2 treated and untreated after viral attachment in Vero cells. (A) Acyclovir control at 10 µg/mL, (B) Virus control (untreated), (C) Cell control, (D) Unripe aqueous extract, (E) Ripe aqueous extract, (F) Unripe ethanolic extract, and (G) Ripe ethanolic extract, all tested at 0.3125 mg/mL

Discussion and Conclusion

- The ethanolic extract of ripe *C. carandas* fruits exhibited the highest extraction yield at 38.46%.
- The aqueous extract of ripe *C. carandas* fruits demonstrated the strongest antioxidant activity, with DPPH and ABTS values of 2.12 ± 0.34 mg GAE/g extract and 29.18 ± 2.21 mg Trolox/g extract, respectively.
- The ethanolic extract of ripe *C. carandas* fruits showed the highest total phenolic content at 8.00 ± 0.58 mg GAE/g extract and it was consistent with the previous study reported higher phenolic level in ethanolic extract (Neimkhum *et al*., 2021)
- The ethanolic extract of unripe *C. carandas* fruits exhibited the lowest cytotoxicity.
- The ethanolic extract of ripe *C. carandas* fruits demonstrated the greatest antiviral activity after viral attachment, achieving 96.47 ± 1.97% inhibition at 0.3125 mg/mL, which may be attributed to its high anthocyanin content, particularly Cyanidin-3-glucoside, reported to inhibit viral replication (Sarkar *et al*., 2018).

Acknowledgements

This research was supported by Assoc. Prof. Dr. Yingmanee Tragoolpua, SCB2711 Laboratory members and the Division of Microbiology, Department of Biology, Faculty of Science, Chiang Mai University.

References

- Mishra, B., Tomer, V., & Kumar, A. (2024). Karonda (*Carissa carandas* L.): A miracle fruit with multifaceted potential. Journal of Agriculture and Food Research, 18, 101417.
- Neimkhum, W., Anuchapreeda, S., Lin, W. C., Lue, S. C., Lee, K. H., & Chaiyana, W. (2021). Effects of *Carissa carandas* Linn. Fruit, pulp, leaf, and seed on oxidation, inflammation, tyrosinase, matrix metalloproteinase, elastase, and hyaluronidase inhibition. Antioxidants, 10(9), 1345.
- Sarkar, R., Kundu, A., Banerjee, K., & Saha, S. (2018). Anthocyanin composition and potential bioactivity of karonda (*Carissa carandas* L.) fruit: An Indian source of biocolorant. Lwt, 93, 673-678.