Inhibition of herpes simplex virus type 2 by the extract of *Clitoria ternatea* flower

Yimthida Wongma * and Yingmanee Tragoolpua

Depatment of Biology, Faculty of Science, Chiang Mai University

E-mail : yimthida.w@gmail.com*

FACULTY OF SCIENCE
CHIANG MAI UNIVERSITY

Abstract

Herpes simplex virus type 2 (HSV-2) is a virus that can cause genital herpes, a sexually transmitted disease. HSV-2 infections are commonly treated with the antiviral drug acyclovir. However, long-term usage of synthetic drug can lead to drug resistant strains of HSV. Therefore, this study is important for exploring flower extracts as an alternative agent that can inhibit HSV-2 infection. The efficacy of extracts from butterfly pea flowers (*Clitoria ternatea* L.) was tested at non-toxic concentrations to determine their ability to inhibit HSV-2. Four extracts of crude and anthocyanin extracts obtained from aqueous and ethanolic extraction were studied. Cytotoxicity was assessed using the MTT assay, which revealed that the aqueous crude extract and anthocyanin that extracted with 1% acetic acid showed 50% cytotoxic concentrations (CD50) of 3.14 ± 0.16 mg/ml and 2.81 ± 0.09 mg/ml, respectively. The ethanolic crude extract and anthocyanin that extracted with 1% acetic acid in 50% ethanol demonstrated CD50 values of 1.43 ± 0.14 mg/ml and 0.72 ± 0.03 mg/ml, respectively. The antiviral activity was assessed using the plaque reduction assay after viral attachment to the cultured cells. The results showed that at a concentration of 1 mg/ml, the anthocyanin that extracted with 1% acetic acid and aqueous crude extract inhibited the virus by 61.44 ± 1.11% and 50.71 ± 4.15%, respectively, while the ethanolic crude extracts inhibited HSV-2 less than 50%. During viral attachment phase, all four extracts inhibited the virus by more than 50%. Specifically, the ethanolic crude extract at concentrations of 1 mg/ml and anthocyanin that extracted with 1% acetic acid in 50% ethanol at concentrations of 0.25 mg/ml inhibited the virus by 88.35 ± 1.51% and 71.16 ± 1.17%, respectively. Therefore, the anthocyanin that extracted with 1% acetic acid and aqueous crude extract of butterfly pea flowers effectively disrupted the virus after attachment to cultured cells. However, the ethanolic extracts and anthocyanin that extracted with 1% acetic acid in 50% ethanol showed greater inhibition during the virus attachment. These results suggest that the development of products for the treatment or prevention of herpes simplex viral infections by anthocyanin and the extract of butterfly pea flowers should be performed in the future.

Introduction

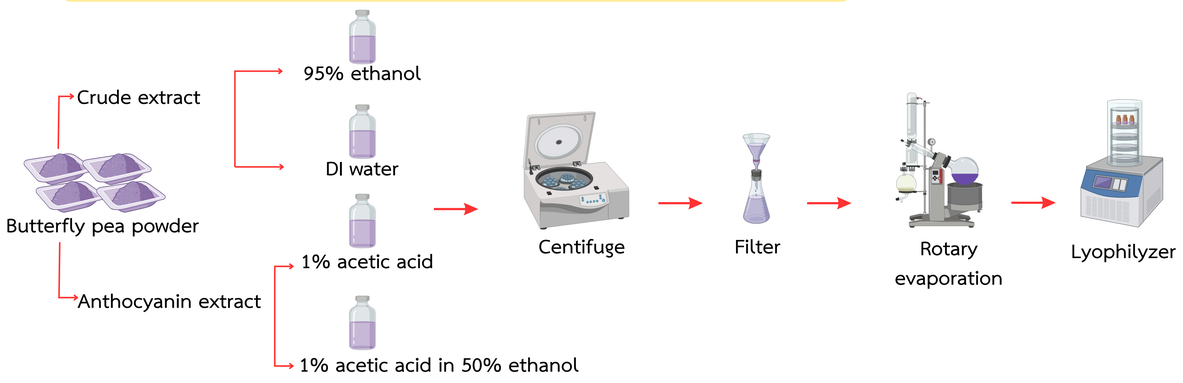
Herpes simplex virus (HSV) infection is transmitted through direct contact with the exudate from herpes lesions, where the virus establishes latency in the nerve ganglia and may be reactivated upon exposure to triggering factors such as stress or immune suppression. HSV is classified into two types. HSV-1 primarily causes oral lesions and HSV-2 predominantly associates with genital infections through sexual transmission. This study explores the efficacy of *Clitoria ternatea* L. (butterfly pea) extract, known for its diverse pharmacological properties, including neuroprotective, anxiolytic, antioxidant, and anti-inflammatory effects. The objective of this study is to evaluate its potential in inhibiting HSV-2 with the aim of developing therapeutic and prophylactic interventions for HSV-related conditions.

Objectives

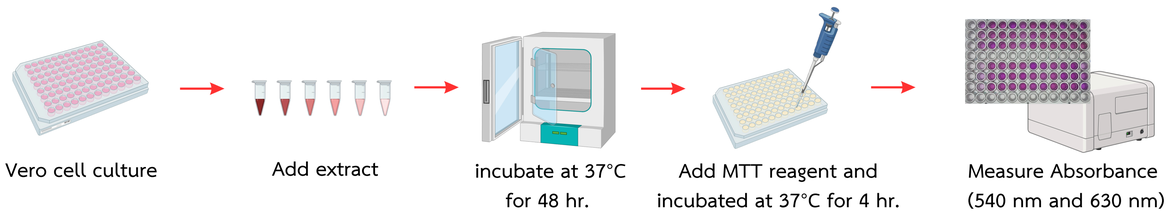
1.To study the toxicity of crude extract and anthocyanin extract from butterfly pea (*Clitoria ternatea* L.) flowers on Vero cells
2.To determine the efficacy of crude extract and anthocyanin extract from butterfly pea (*Clitoria ternatea* L.) flowers against herpes simplex virus type 2 (HSV-2)

Methodology

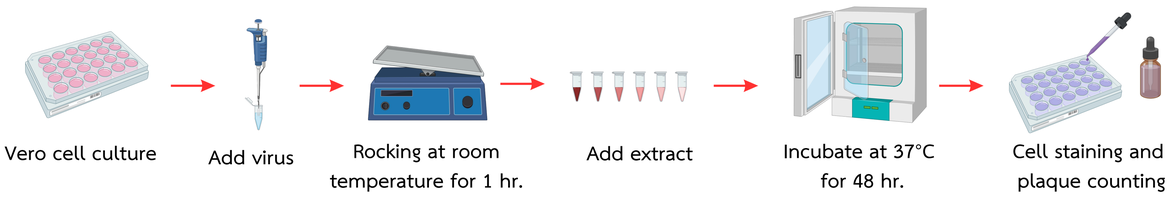
1 Extraction of compounds from butterfly pea (*Clitoria ternatea* L.) flowers



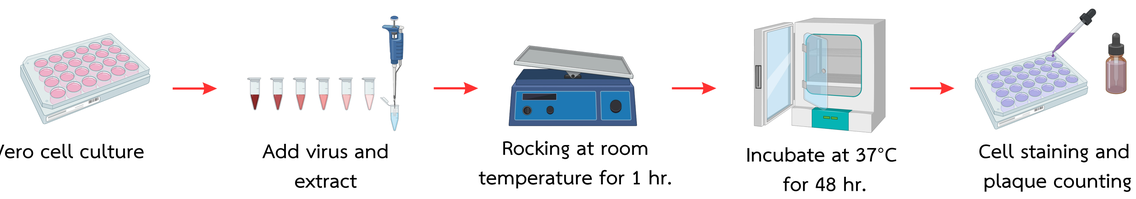
2 Cytotoxicity testing of butterfly pea extracts by the MTT assay



3 Determination of the inhibitory effect of butterfly pea extracts on HSV-2 after viral attachment



4 Determination of the inhibitory effect of butterfly pea extracts on HSV-2 during viral attachment



5 Analysis of total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TA) and antioxidant activity testing (DPPH assay and ABTS assay)

Acknowledgement

This research was supported by Assoc. Prof. Dr.Yingmanee Tragoolpua, the member in the 2711 lab, and Division of Microbiology, Department of Biology, Faculty of Science, Chiang Mai University.

Results

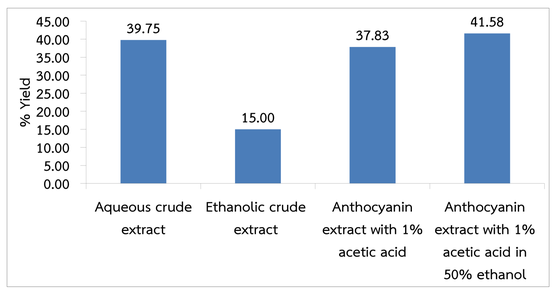


Fig 1. The yield of *Clitoria ternatea* flower extract

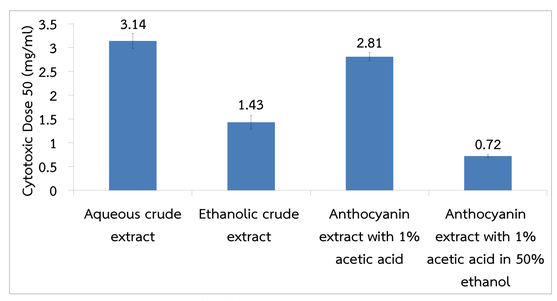


Fig 2. Cytotoxicity test

Table 1. Anti-HSV-2 activity of *Clitoria ternatea* flower extract

Types of extracts	Solvent	Concentration (mg/ml)	Inhibition (%) After virus attachment	Inhibition (%) During virus attachment
Crude extract	Aqueous	0.25	14.25 ± 4.08	22.78 ± 2.12
		0.50	38.54 ± 4.69	36.03 ± 1.57
		1.00	50.71 ± 4.15	62.07 ± 1.92
	Ethanol	0.25	13.07 ± 2.41	39.73 ± 0.15
		0.50	35.08 ± 0.80	54.40 ± 0.23
		1.00	45.48 ± 3.25	88.35 ± 1.51
Anthocyanin extract	1% acetic acid	0.25	12.11 ± 1.42	31.17 ± 2.67
		0.50	32.77 ± 2.34	47.90 ± 0.66
		1.00	61.44 ± 1.11	66.34 ± 2.90
	1% acetic acid in 50% ethanol	0.0625	15.67 ± 1.27	23.78 ± 1.28
		0.125	22.88 ± 0.22	39.50 ± 1.93
		0.25	38.69 ± 1.69	71.16 ± 1.17

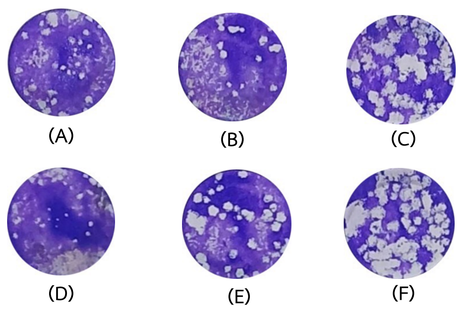


Fig 3. Viral plaques when treatment by aqueous crude extract (A), anthocyanin extract with 1% acetic acid (B), ethanolic crude extract (D), anthocyanin extract with 1% acetic acid in 50% ethanol (E) after viral attachment comparing to control viruses (C) & (F).

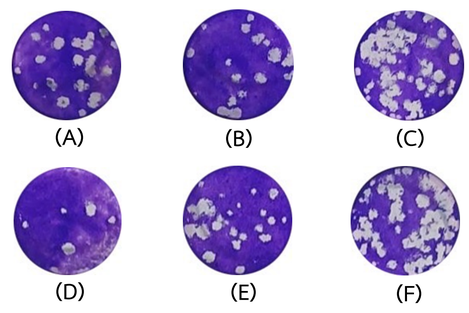


Fig 4. Viral plaques when treatment by aqueous crude extract (A), anthocyanin extract with 1% acetic acid (B), ethanolic crude extract (D), anthocyanin extract with 1% acetic acid in 50% ethanol (E) during viral attachment comparing to control viruses (C) & (F).

Table 2. Analysis of antioxidant content and testing of antioxidant activity

Types of extracts	Solvent	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	Anthocyanin (mg/g)	DPPH (mg GAE/g extract)	ABTS (mg trolox/g extract)
Crude extract	Aqueous	23.00 ± 1.35	25.84 ± 0.61	1.31 ± 0.02	13.10 ± 1.07	43.90 ± 1.23
	Ethanol	19.18 ± 1.29	33.61 ± 1.98	ND	4.02 ± 0.58	23.93 ± 1.36
Anthocyanin extract	1% acetic acid	19.71 ± 0.10	24.05 ± 0.68	1.23 ± 0.03	6.13 ± 0.21	47.40 ± 8.68
	1% acetic acid in 50% ethanol	23.96 ± 0.63	29.60 ± 1.19	0.76 ± 0.01	9.12 ± 0.95	35.47 ± 5.32

Discussion & Conclusion

- Butterfly pea flowers extracted with 1% acetic acid in 50% ethanol demonstrated the highest production of anthocyanin.
- Aqueous crude extract exhibited the lowest toxicity compared to the other extracts.
- Anthocyanin extracted with 1% acetic acid provided the most effective viral inhibition after virus attachment. The extract demonstrates interactions or properties that inhibit post-attachment processes, such as suppressing HSV replication and reducing viral protein expression (Sivarajan *et al*., 2022).
- Ethanolic crude extract demonstrated the highest efficacy in inhibiting during virus attachment. The bioactive compounds in the ethanolic extract may interact with viral surface proteins, preventing the virus from entering host cells (Suantai *et al*., 2022).
- The anthocyanin extracted with 1% acetic acid in 50% ethanol also exhibited the highest phenolic content. However, The ethanolic crude extract contained the highest flavonoid content. The highest anthocyanin content was observed from the aqueous extract of butterfly pea flowers.

References

Nithianantham, K., Shyamala, M., Chen, Y., Yoga Latha, L., Jothy, S. L., & Sasidharan, S. (2011). Hepatoprotective potential of *Clitoria ternatea* leaf extract against paracetamol-induced damage in mice. Molecules, 16, 10134-10145; doi:10.3390/molecules161210134

Sivarajan, R., Oberwinkler, H., Roll, V., König, E. M., Steinke, M., & Bodem, J. (2022). A defined anthocyanin mixture sourced from bilberry and black currant inhibits measles virus and various herpesviruses. BMC Complementary Medicine and Therapies, 22(1), 161; doi:10.1186/s12906-022-03661-7

Suantai, B., Jantakee, K., Kaewkod, T., Sangboonruang, S., Chitov, T., & Tragoolpua, Y. (2022). Anthocyanins in red jasmine rice (*Oryza sativa* L.) extracts and efficacy on inhibition of herpes simplex virus, free radicals, and cancer cells. Nutrients, 14, 1905; doi:10.3390/nu14091905