Inhibition of Herpes simplex virus type 2 by Kaffir lime and Lime leaf extracts

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Abstract

Herpes Simplex Virus Type 2 (HSV-2) is a primary causative agent of genital herpes and significantly impacts the quality of life of affected individuals. While conventional treatment relies on antiviral drugs. The emergence of drug-resistant strains and the adverse effects associated with long-term therapy have necessitated the search for effective natural alternatives. This study aimed to evaluate the inhibitory effects of *Citrus hystrix* (Kaffir lime) and *Citrus aurantifolia* (Lime) leaf extracts against HSV-2 using plaque reduction assay. The antioxidant activity assessment via DPPH assay revealed that the ethanolic extract of lime leaves exhibited the highest potency at a concentration of 1.547 ± 0.083 mg/ml. The ABTS assay identified the aqueous extract of lime leaves as the most effective, with an antioxidant activity of 1.845 ± 0.111 mg/ml. Analysis of total phenolic content indicated that the ethanolic extract of Kaffir lime leaves contained the highest concentration of 36.528 ± 6.004 mg gallic acid/g extract. Cytotoxicity testing on Vero cells demonstrated that the aqueous extract of kaffir lime leaves possessed the lowest toxicity, with a Cytotoxic Dose (CD_{50}) of 26.44 ± 0.29 mg/ml. Regarding the antiviral mechanisms, the results during the post-attachment phase showed that aqueous extracts of kaffir lime and lime leaves achieved maximum viral inhibition of 86.95 and 100 at 5.00 mg/ml, respectively. Meanwhile, ethanolic extracts of kaffir lime and lime leaves reached peak inhibition levels of 48.44 and 65.45 at 0.078 mg/ml, respectively. These findings suggest that both *Citrus hystrix* and *Citrus aurantifolia* leaf extracts possess significant potential as natural agents for inhibiting HSV-2.

Introduction

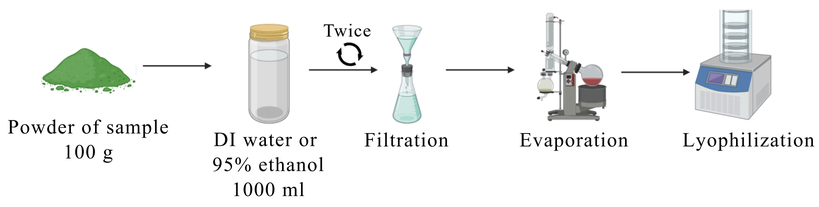
Herpes is a chronic viral infection caused by the Herpes Simplex Virus (HSV), a member of the Herpesviridae family. HSV-2 (Herpes Simplex Virus type 2) is transmitted through direct contact with an infected person or through bodily fluids, such as saliva, fluid from blisters, or the sharing of personal items, as well as contact with lesions in the oral or genital areas. Transmission frequently occurs through sexual intercourse. After infection, patients typically present with cutaneous symptoms, including clear blisters, redness or a burning sensation at the site of infection. These symptoms usually persist for 2–14 days before gradually improving. However, the virus is not completely eliminated from the body. Instead, it remains latent in the ganglia of the peripheral nervous system. It can be reactivated when the body is under stress, when the immune system is weakened, or by other triggering factors. Current guidelines for treating herpes focus on the use of antiviral drugs, such as acyclovir, valacyclovir, and famciclovir. These medications work by inhibiting viral DNA replication, thereby reducing the viral load and alleviating clinical symptoms. Although these drugs are highly effective, they may cause side effects such as nausea and vomiting. Consequently, there is a necessity for the research and development of natural extracts that offer high safety profiles to serve as alternative or complementary treatments for Herpes Simplex Virus infections.

Objective

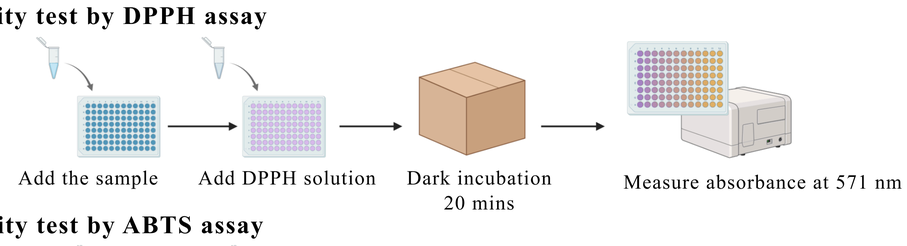
- To investigate the cytotoxicity of Kaffir lime and Lime leaf extracts on Vero cell cultures.
- To evaluate the inhibitory efficacy of Kaffir lime and Lime leaf extracts against Herpes Simplex Virus type 2 (HSV−2) infection.
- To determine the antioxidant properties of the Kaffir lime and Lime leaf extracts.

Methodology

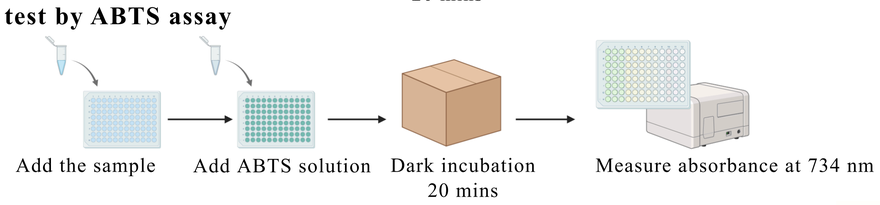
Extraction of compounds from Kaffir lime and Lime leaf



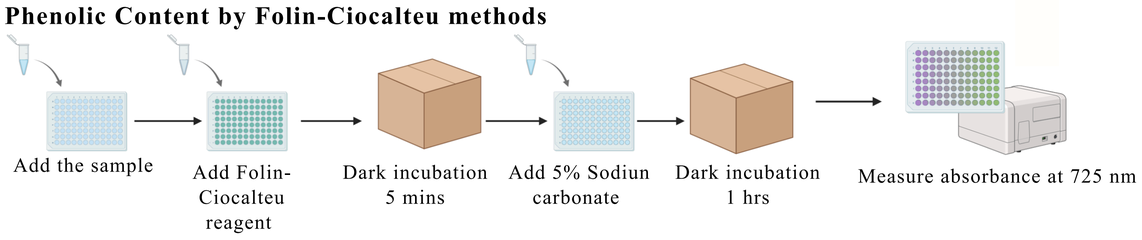
Antioxidant activity test by DPPH assay



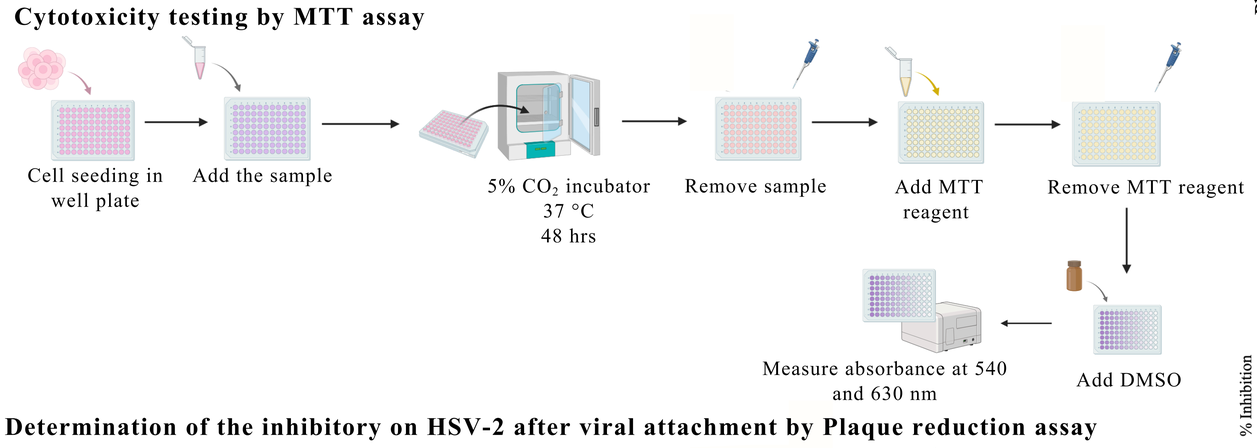
Antioxidant activity test by ABTS assay



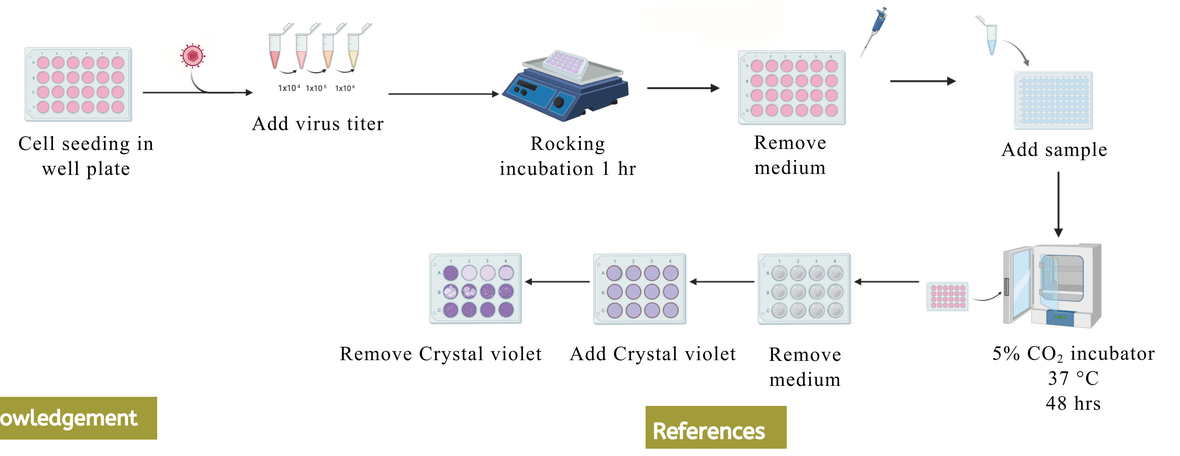
Total Phenolic Content by Folin-Ciocalteu methods



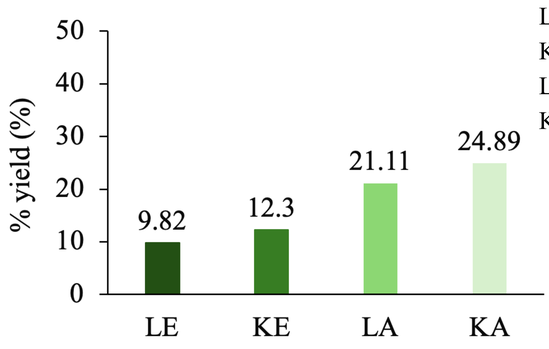
Cytotoxicity testing by MTT assay



Determination of the inhibitory on HSV-2 after viral attachment by Plaque reduction assay



Results



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LE = Ethanolic Lime leaf extract
KE = Ethanolic Kaffir lime leaf extract
LA = Water Lime leaf extract
KA = Water Kaffir lime leaf extract

Figure 1 Quantity of extracts

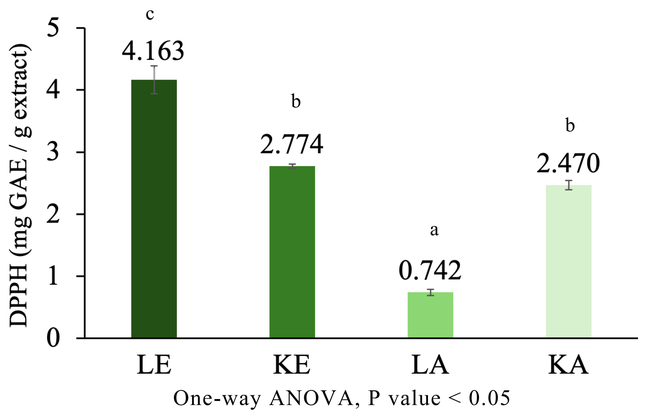


Figure 2 Antioxidant of kaffir lime and lime leaf extracts by DPPH assay

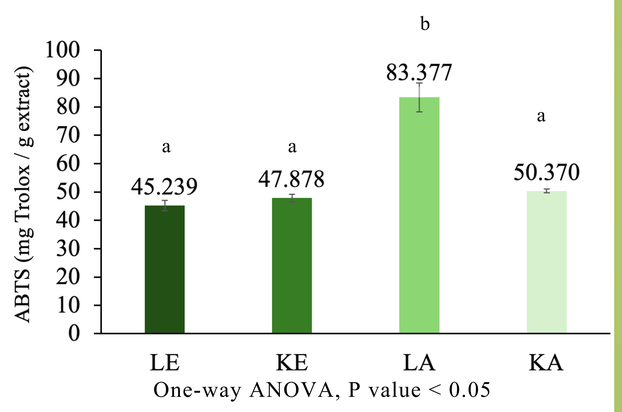


Figure 3 Antioxidant of kaffir lime and lime leaf extracts by ABTS assay

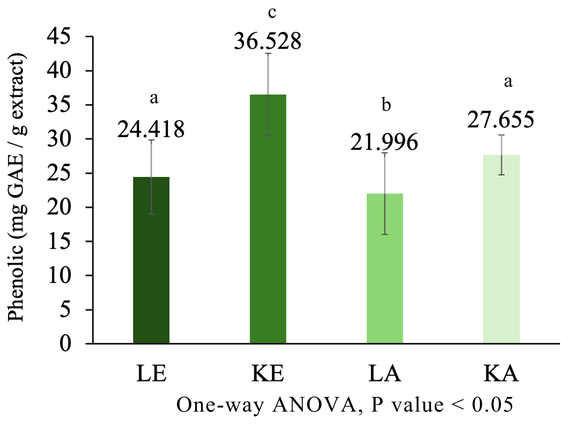


Figure 4 Total Phenolic Content by Folin-Ciocalteu methods

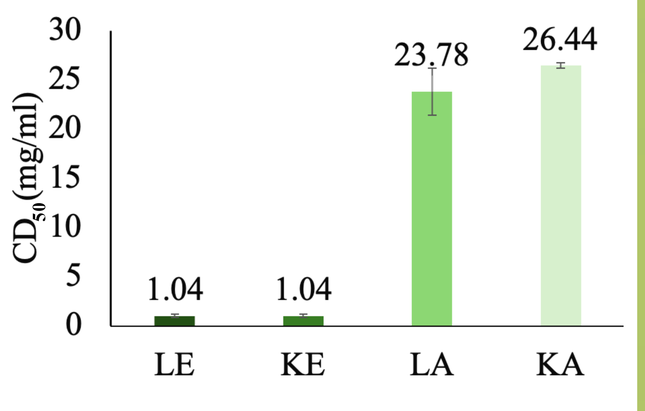


Figure 5 Cytotoxicity testing by MTT assay

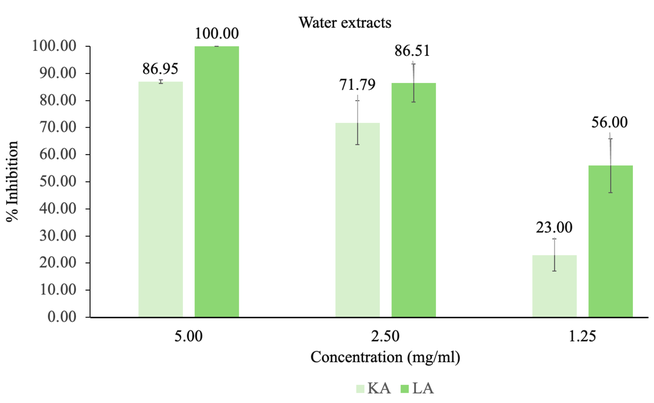


Figure 6 Inhibition after viral attachment by water extracts

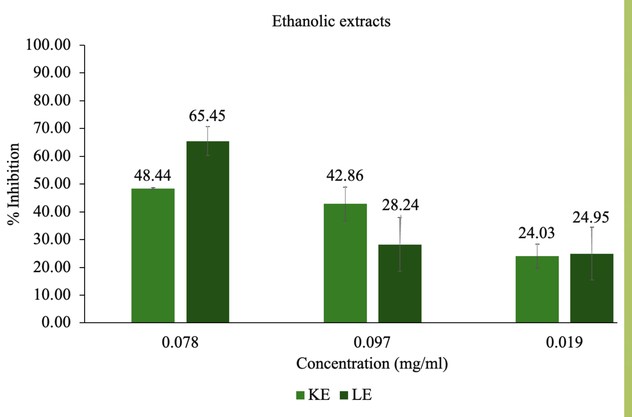


Figure 7 Inhibition after viral attachment by ethanolic extracts

Conclusions

- Water Kaffir lime leaf extract yielded the highest value and exhibited the lowest toxicity compared to the other extracts.
- Ethanolic Kaffir lime leaf extract had the highest phenolic content at 36.53 ± 6.00 mg GAE/g extract.
- Ethanolic Lime leaf extract had the highest DPPH at 4.16 ± 0.23 mg GAE/g extract.
- Water Lime leaf extract had the highest ABTS at 83.38 ± 5.04 mg Trolox/g extract. and inhibited the virus most effectively after virus attachment at a concentration of 5.0 mg/mL, achieving 100.00% inhibition.

Acknowledgement

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- 2711 Laboratory

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