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Research Article

Antiviral Activity of an Extract from Leaves of the Tropical Plant *Cynometra cauliflora*

Noor Zarina Abd Wahab[1,*], Aziah Azizul[1], Norhidayah Badya[2], Nazlina Ibrahim[3]

ABSTRACT

Background: *Cynometra cauliflora* is a species of tree in the family Fabaceae and has been used in folk medicinal preparation. **Objectives:** In this study, *Cynometra cauliflora* methanolic leaves extract was tested against clinical isolate herpes simplex virus type-1 (HSV-1). **Materials and Methods:** The leaves of *C. cauliflora* plant was extracted using methanol extraction method. Cytotoxicity was assessed using 3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) assay. Plaque reduction assays were carried out to evaluate the antiviral activity of *C. cauliflora* extract against HSV-1. These include post-treatment, pre-treatment and virucidal assays. **Results:** The value of cytotoxic concentration, CC_{50} of *C. cauliflora* extract was 36 mg/mL. High antiviral activity was observed in post-treatment. *C. cauliflora* extract treatment was found to not interfere directly to infectious particle and confer mild protection when given as prophylaxis. **Conclusion:** This study provides important novel insights on the phytomedicinal properties of *C. cauliflora* extracts on HSV-1.

Key words: Herpes simplex virus type 1, *Cynometra cauliflora*, plaque reduction assay, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); virucidal.

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INTRODUCTION

Medicinal plants of the Malaysian forest were reportedly rich in biological activities. Several interesting natural products were isolated from local medicinal plants such as styrylpyrone derivatives isolated from *G. umbrosus* have shown a potent antiviral activity against HSV-1 and dengue virus type 2 (DENV-2).[1-3] Other *in vitro* studies also showed SPD is active against several cancer cell lines namely; HL-60 (leukemia), HepG2 (liver), PANC-1 and Hela cells,[4-5] geraniin extracted from the rind of *Nephelium lappaceum* that were reported has been shown to exhibit antiviral properties against several types of viruses, as well as crude methanolic extract of *Psidium guajava* leaves extract that were reported to have antibacterial activities against foodborne pathogens.[6] This plant extract also was reported to have antiviral activity against DENV-2.[7] Previous studies have shown antioxidant, antiinflammatory, antitumor, antimicrobial and antidiabetic activity of *C. cauliflora.*[8-10] *C. cauliflora* L. or commonly known as 'Nam-Nam' among native Malaysian is a tropical plant under the Fabaceae family. It is also commonly known by local as NamNam or Buah Katak Puru in Malaysia.[11] The fruit which are kidney-shape pod, greenish yellow to brown, with a sandy and wrinkled surface. It can be consumed as fruit salad (ulam).[12]

Herpes Simplex Virus type-1 (HSV-1) is a common pathogen which causes cold sores or common cold and orolabial infection. Normal sites of infection are mucosal epithelium, hence keratitis labial herpes, gingivostomatitis, and genital herpes. Infection can disseminate from mucosal epithelium to other tissues with slow healing and more detrimental outcome in immunocompromised individual.[13] This includes in newborn babies, transplant patient or HIV patient who are readily struggling with immature immunity, immune suppressive drugs regiment and prolonged toxicity and prophylaxis, respectively.[14] Generally, HSV-1 infections can be treated successfully with acyclovir. However, drug resistant variants emerged as a result of long-term treatment of immunocompromised patients with acyclovir. This subsequently led to treatment failure.[15] Thus, a new target is required to ensure alternative possible treatments for HSV-1 resistant strains. In order to combat this resistant HSV-1 strain, new antiviral agents with different mode of actions are indeed important. Therefore, the aim of this study was to investigate the potential of crude methanolic extract of *C. cauliflora* leaves as an antiviral agent against HSV-1 infection.

MATERIALS AND METHODS

Plant material

The fresh leaves parts were collected from the state of Terengganu, Malaysia. The leaves were cleaned with tap water to remove dirt and oven-dried at 60°C. Dried leaves powder of *C. cauliflora* was extracted with methanol. *C. cauliflora* leaves (100 g) was macerated with methanol (300 mL) to produce crude methanol extract. The extracts were filtered and solvent was evaporated under reduced pressure using rotary vacuum evaporator.

Cells and virus

Vero cell from American Type Culture Collection (ATCC) CCL-81 was used for both cytotoxicity and antiviral test. Dulbecco's Modified Eagle's Medium

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(DMEM) (SigmaAldrich, USA) supplemented with 5% fetal bovine serum (FBS) (Sigma- Aldrich, USA) was used for cell maintenance throughout the experiment. Clinical strain of HSV-1 used was obtained from the stock culture of Faculty Science and Technology, Universiti Kebangsaan Malaysia.

Cytotoxicity test

Briefly, Vero cells (2.5×10^5 cells/mL) were seeded into 96-well plates and incubated overnight at 37°C. Upon 80% confluence, the cells were treated with several concentrations of extract, ranging from 3.13 mg/mL to 100 mg/mL. After incubation of about 72h, the growth medium was discarded and replaced with 100 µL of MTT solution and incubated for 3h. After that, the MTT solution was discarded, and formazan crystal was dissolved using 100 µL of dimethyl sulphoxide (DMSO) to lyse the cells. Colour development was detected using a microplate reader (TECAN Infinite 200 PRO, Austria) at 540 nm. Optical density (OD) of individual well was quantified using spectrophotometer at 540nm.[16] Cells viability was calculated using formula below:

Cell viability (%) = ODtest – ODblank / ODcell – ODblank x 100

where ODtest = optical absorbance of cells treated with SPD, ODblank = optical absorbance for well filled with DMSO and OD-cells = optical absorbance for cells without treatment with SPD. Nonlinear regression was done to obtain the CC50 value (cytotoxic concentration which killed 50% of cells).

Antiviral assay

Antiviral activity was also evaluated by the plaque assay method. Screening for antiviral activity was performed using 3 different treatments.[17] 1) Post-treatment: To evaluate antiviral activity of extract against intracellular replication of DENV-2, cells were inoculated with virus 2 hour before treatment with extract. 2) Pre-treatment: In order to determine the prophylactic anti-HSV-1 activity of extract, virus was inoculated to cells 24 hours after treatment with extract. 3) Virucidal: Direct virucidal effect of the extract was investigated by incubating virus with extract for 1 hour before it was inoculated on the cells. For the antiviral tests, the extract concentration tested was twice lower than the CC_{50} value in order to reduce the possibility of toxicity towards the cells. The viral concentration used for cell inoculations was fixed at 50 PFU. The effectiveness of extract as an antiviral agent expressed as selectivity index (SI).

Selectivity Index (SI) = Cytotoxicity concentration (CC_{50}) / Effective concentration (EC_{50})

RESULTS

Cytotoxicity evaluation of *C. cauliflora* extract

MTT assay was conducted to determine the cytotoxicity of *C. cauliflora* extract towards Vero cells. The cytotoxicity assay result, as presented in Figure 1, shows the percentage of cell viability versus *C. cauliflora* extract concentration. The estimated CC_{50} value towards the Vero cells was 36.0 mg/mL.

Anti-HSV-1 activity of *C. cauliflora*

Plaque reduction assays were done to screen for anti-HSV-1 activity using *C. cauliflora* extract with different concentrations. Figure 2A, 2B and 2C shows the percentage of plaque reduction in post-treatment, pre-treatment and virucidal assays, respectively. The results from post-treatment assay showed that 100% plaque reduction was achieved at the concentration of 18 mg/mL. In pre-treatment assay, more than 50% plaque reduction was observed at 9 mg/mL. Meanwhile, *C. cauliflora* extract at any concentrations had no virucidal effect on HSV-1.

Effectiveness of certain compounds or extracts can be evaluated by using selective index (SI). In post-treatment assay, *C. cauliflora* extract exhibited potent antiviral activity against HSV-1 with EC_{50} = 2.14 mg/mL and with SI value of 16.8 (Table 1). Pre-treatment of Vero cells with *C. cauliflora* extract exhibited the prophylactic activity of extract against HSV-1 infection with EC_{50} = 8.5 mg/mL and with SI value of 4.23 (Table 1). *C. cauliflora* extract when added simultaneously with the virus not showed any anti-adsorption activity against HSV-1 (Table 1). Result revealed that *C. cauliflora* extract had greater SI value in post-treatment. Any antimicrobial compound that has SI values higher than 10 (SI>10) ensures the potential to be developed as an agent of antiviral drug.[18] Selectivity index of *C. cauliflora* extract against HSV-1 was more than 10 indicating potential as antiviral agent.

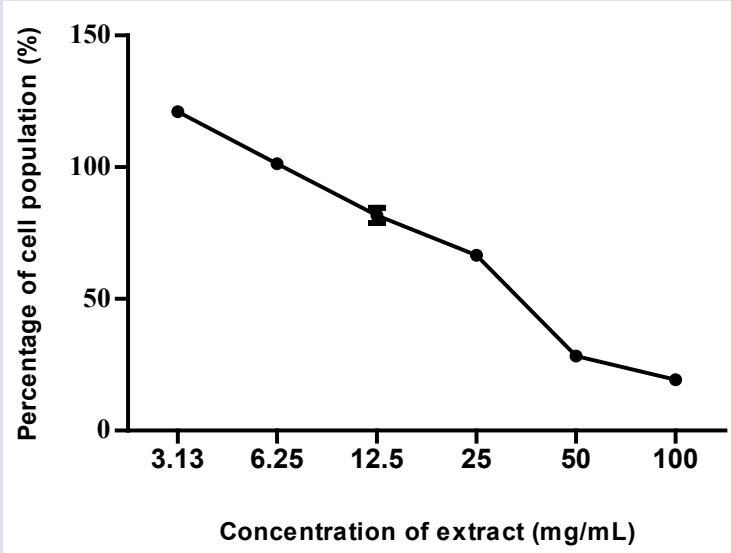
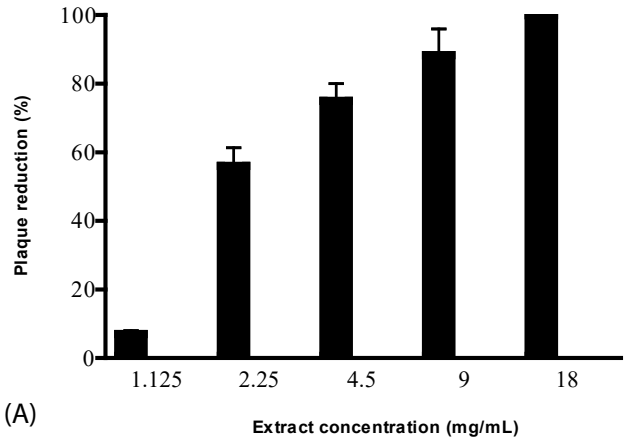
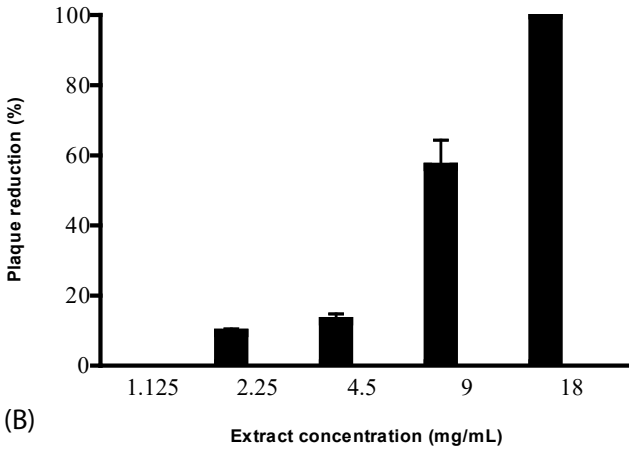


Figure 1: Effect of different concentration of *C. cauliflora* extracts towards population of Vero cells.





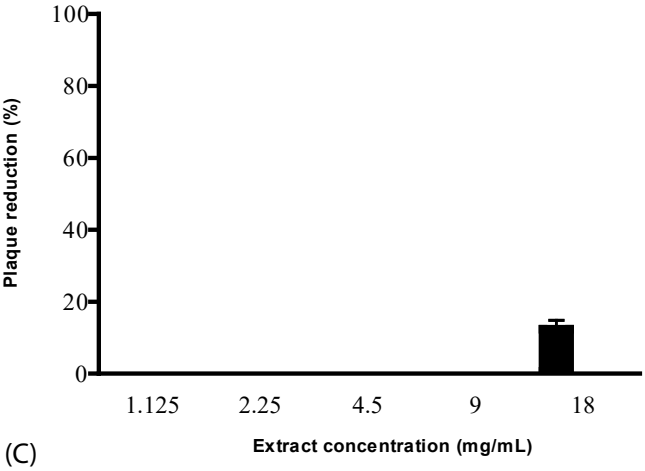


Figure 2: Effect of post-treatment (A), pre-treatment (B) and virucidal (C) of *C. cauliflora* extract on HSV-1 plaque reduction.

Table 1: CC_{50}, EC_{50} and SI values of all extracts in post-treatment assay, pre-treatment assay and virucidal assay.

	CC_{50} (mg/mL)	EC_{50} (mg/mL)	SI (CC_{50}/EC_{50})
Post-treatment	36.0	2.14	16.8
Pre-treatment	36.0	8.5	4.23
Virucidal	36.0	-	-

CC_{50}: Cytotoxic concentration of SPD; EC_{50}: Effective concentration of SPD; SI: Degree of selectivity.

DISCUSSION

Based on phytochemical analyses the findings in previous study, *C. cauliflora* leave extract has been reported to be rich in secondary metabolites such as tannin, flavonoid, saponins, cardiac glycosides and terpenoids.[19] Lyu and collaborators[20] reported the elucidation of the mechanism of the antiherpetic (HSV-1) activity *in vitro* via plaque reduction assay of flavonoid. Similarly, Sieniawska[21] demonstrated that tannins and related compounds, exhibit antiherpes activity *in vitro*. In addition, Perez[22] reported that saponins inhibit the replication of HSV-1 and poliovirus type 2 as shown by inhibition of cytopathic effect and reduction of virus production. Thus, the richness of secondary metabolites in *C. cauliflora* plant may contribute to anti-HSV-1 properties. In this study, we investigated whether *C. cauliflora* methanolic extracts could confer protection to cells before or after the initiation of HSV-1 infection. The ability of the extract to act directly against HSV-1 virion particle was observed in virucidal assay. This antiviral analysis was performed on Vero cells as a model of infection in mammalian cells.

Screening for antiviral activity involves post-, pre- and virucidal treatment to determine the best mode for antiviral administration. In this part of the study, *C. cauliflora* extract treatment was found to not interfere directly to infectious particle and confer mild protection when given as prophylaxis. Instead, evidence showed that extract-HSV-1 treatment most effective when administered as post-treatment. *C. cauliflora* extract anti-HSV-1 activity was observed to be concentration dependent. The ability of *C. cauliflora* to confer protection to the cells before HSV-1 infection was tested by pretreating the cells with *C. cauliflora* methanol extracts for 24 h prior to viral infection. Protection could be conferred through extracellular mechanisms. The *C. cauliflora* extracts might interrupt the interaction of several envelope glycoproteins with cell surface receptors requires for fusion of the virion envelope with a cell plasma membrane, resulting in ineffective viral infection.[23] Pre-treatment was done to study the effect of the extract as prophylactic agent in protecting the cell from HSV-1 adsorption and penetration. *C. cauliflora* extracts presented low to mild prophylactic effects, perhaps due to the presence of various plant alkaloids in the crude extract of *C. cauliflora*, which may act synergistically to decrease the effective interaction of the active compounds. Additionally, the results are presented as some of the antiviral compounds in these extracts may be present at low levels in a non-cytotoxic dilution of the extract.[24] Therefore, extract can act as partial prophylactic agent to protect Vero cells against HSV-1 infection. Virucidal agents are chemical substances that attack and inactivate the extracellular viral particles by damaging the protein coat or penetrating the virion or by destroying the viral genome resulting in decreased infectivity of the virus.[25] The possibility of this occurring was demonstrated using a virucidal assay. *C. cauliflora* extract treatment was found to not interfere directly to infectious particle because no inhibition was observed.

CONCLUSION

As a conclusion, our findings suggest that crude extract prepared from *C. cauliflora* contains antiviral active compounds and could be potential antiviral agent.

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CONFLICTS OF INTEREST

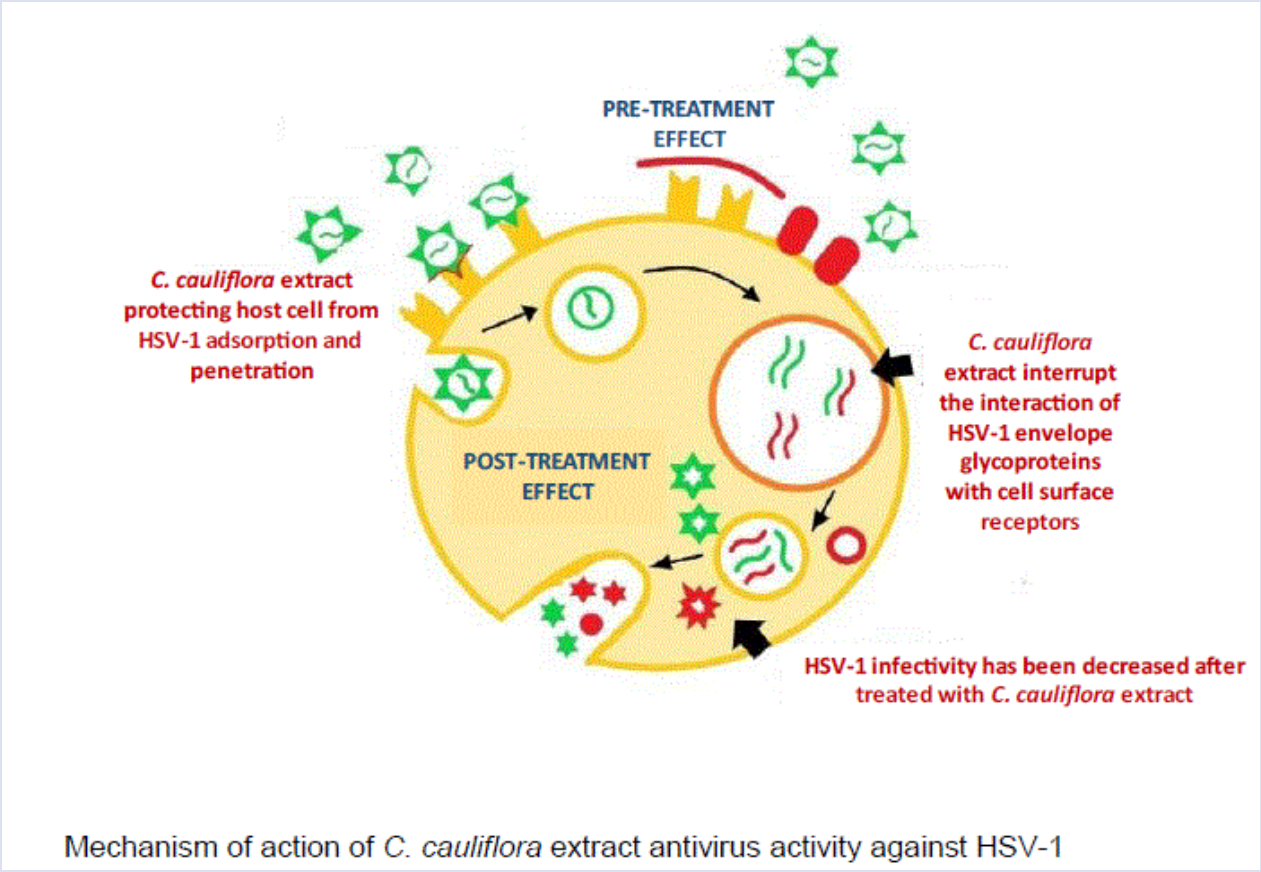
None.

REFERENCES

1. Md Nor NS, Ibrahim N. Styrylpyrone derivative of *goniothalamus umbrosus* inhibit HSV-1 infection during viral early replication cycle. Antiviral Research. 2011;90:A21-78.
2. Ibrahim N, Shahar S, Wahab NZA, Nor NSM. Effect of styrylpyrone derivative (SPD) and SPD/foscarnet combination towards virus infected cell. The 2018 UKM FST Postgraduate Colloquium AIP Conf. Proc. 2019;2111:040002-1-4.
3. Noor Zarina AW, Nazlina I. *In vitro* study, antiviral activity of styrylpyrone derivative against dengue virus type 2. Asian Journal of Plant Sciences 2020;19:438-42.
4. Inayat-Hussain SH, Annuar BO, Din LB, Ali AM, Ross D. Loss of mitochondrial transmembrane potential and caspase-9 activation during apoptosis induced by the novel styryl-lactone goniothalamin in HL-60 leukemia cells. Toxicology *in vitro*: An International Journal Published in Association with BIBRA. 2003;17(4):433-9.
5. Hawariah A, Stanslas J. *In vitro* response of human breast cancer cell lines to the growth-inhibitory effects of styrylpyrone derivative (SPD) and assessment of its antiestrogenicity. Anticancer Res. 1998;18(6A):4383-6.
6. Nurul Alia A, Noor Zarina AW, Noor Ayunie M, Azlin SS, Ahmad Syibli O. Antimicrobial Activity of *Psidium guajava* Leaves Extract Against Foodborne Pathogens. International Journal of Psychosocial Rehabilitation. 2020;24(7):2020.
7. Noor Zarina AW, Norhidayah B, Nazlina I, Mohd Khairul Amri K, Hafizan J, *et al.* Antiviral Activity of *Cynometra Cauliflora* Leaves Methanolic Extract Towards Dengue Virus Type 2. International Journal of Engineering & Technology. 2018;7 (3.14):344-7.
8. Abd Aziz AF, Iqbal M. Antioxidant Activity and Phytochemical Composition of *Cynometra cauliflora*. J Exp Integr Med. 2013;3(4):337-41.
9. Tajudin TJ, Nashriyah Mat SA, Siti-Aishah AB, Aziz A, Yusran M, Alwi A, *et al.* Cytotoxicity, antiproliferative effects, and apoptosis induction of methanolic extract of *Cynometra cauliflora* Linn. Whole fruit on human promyelocytic leukemia HL-60 cells. Evidence-Based Complementary and Alternative Medicine. 2012;1-6.
10. Khoo HE, Azlan A, Kong KW, Ismail A. Phytochemicals and Medicinal Properties of Indigenous Tropical Fruits with Potential for Commercial Development. Evidence-Based Complementary and Alternative Medicine. 2016;1-20.
11. Marbawi H, Sidi Ahmad SN, Baharudin NS, Gansau JA. *In vitro* embryo germination and callus induction of *Cynometra cauliflora*, an underutilized medicinal plant. Transactions on Science and Technology. 2016;3(3):476-82.
12. Lim TK. Edible Medicinal and Non-Medicinal Plants. 2012.
13. Horbul JE, Schmechel SC, Miller BRL, Rice SA, Southern PJ. Herpes simplex virus-induced epithelial damage and susceptibility to human immunodeficiency virus type 1 infection in human cervical organ culture. PLoS ONE 2011;6(7):e22638.
14. Shin H, Iwasaki A. Generating protective immunity against genital herpes. Trends in Immunology 2013;34(10):487-94.
15. Krawczyk A, Arndt MAE, Grosse-Hovest L, Weichert W, Giebel B, Dittmer U, *et al.* Overcoming drug-resistant herpes simplex virus (HSV) infection by a humanized antibody. Proceedings of the National Academy of Sciences. 2013;110(17):6760-5.
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65(1-2):55-63.
17. Moses M, Md. Nor, NS, Ibrahim N. *In vitro* virucidal activity of a styrylpyrone derivative against herpes simplex virus strain KOS-1. AIP Conference Proceedings. 2014;1614562.
18. Dargan DJ. Methods in Molecular Medicine. 1998;Totowa, New Jersey: Humana Press.

19. Noor Zarina AW, Norhidayah B, Nazlina I, Mohd Khairul Amri K. Phytochemistry and antibacterial activity of *Cynometra Cauliflora*. Indian Journal of Public Health Research & Development. 2019;10(04):765-9.
20. Lyu S-Y, Rhim J-Y, Park W-B. Antiherpetic activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) *in vitro*. Arch Pharm Res. 2005;28(11):1293-301.
21. Sieniawska E. Activities of Tannins-from *in vitro* Studies to Clinical Trials. Natural Product Communications. 2015;10(11),1934578X1501001.
22. Perez RM. Antiviral Activity of Compounds Isolated from Plants. Pharmaceutical Biology. 2003;41(2):107-57.
23. Antoine TE, Park PJ, Shukla D. Glycoprotein targeted therapeutics: a new era of anti-herpes simplex virus-1 therapeutics. Reviews in Medical Virology. 2013;23(3):194-208.
24. Semple SJ, Reynolds GD, O'Leary MC, Flower RLP. Screening of Australian medicinal plants for antiviral activity. J Ethnopharmacol. 1998;60:163-72.
25. Galabov AS. Virucidal agents in the eve of manorapid synergy. GMS Krankenhhyg Interdiszip, 2007;2(1):18.

GRAPHICAL ABSTRACT



Mechanism of action of *C. cauliflora* extract antivirus activity against HSV-1

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