

Inhibition of Herpes Simplex Virus Type 2 *In Vitro* by Durian (*Durio zibethinus* Murray) Seed Coat Crude Extracts

Jiraporn Nikomtat^{1,a*}, Pattachai Pinnak^{1,b}, Kodchakorn Lapmak^{1,c},
Pathompong Tammalungka^{2,d}, Thitiphorn Thiankhanithikun^{1,e},
and Yingmanee Tragoolpua^{3,f}

¹Faculty of Science and Technology, Uttaradit Rajabhat University, Uttaradit, Thailand

²Faculty of Humanities and Social Sciences, Uttaradit Rajabhat University, Uttaradit, Thailand

³Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

^{a,*}nikomtat_jk@hotmail.com, ^bbiology_sc25@hotmail.com,

^ckodchakorn.lapmak@gmail.com, ^dskymanphare7589@gmail.com, ^etiphron@hotmail.com,
^fyingmanee.t@cmu.ac.th

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Abstract. The aim of this study was to determine Herpes simplex virus type 2 (HSV-2G) inhibition by durian seed coat crude extracts. Dried native durian seed coat from Lab-Lae District, Uttaradit Province was extracted with solvents; distilled water and ethanol. Subsequently, two durian seed coat crude extracts were investigated their cytotoxicity on Vero cells and determined HSV-2G inhibition by plaque reduction assay. The results displayed that 50% cytotoxicity dose (CD₅₀) of aqueous and ethanolic extracts of durian seed coat were 224.40 and 112.47 µg/ml, respectively. Moreover, the aqueous and ethanolic extracts of durian seed coat at a concentration of 100 µg/ml could inhibit HSV-2G by 36.34 ± 1.47 and 61.56 ± 5.30%, respectively. This study displayed the one of advantages of native seed durian in Uttaradit.

Introduction

Herpes simplex virus (HSV) have been classified into subfamily *Alphaherpesvirinae*, which having variable host range, relatively short reproductive cycle, rapidly spreading in culture, efficient destruction of infected cells, and capacity to establish latent infections in sensory ganglia. This subfamily contains the genera *Simplexvirus* (HSV-1, HSV-2, circopithecine herpesvirus1, bovine mamillitis virus), *Varicellovirus* (VZV), psuedorabies virus and equine herpesvirus1. Moreover, herpesvirus appear to share four significant biological properties. First, all herpesvirus have a large array of enzymes involved in nucleic acid metabolism such as thymidine kinase, thymidylate synthetase, dUTPase and ribonucleotide reductase. DNA synthesis enzyme included DNA polymerase, helicase, primase and protein kinase involved processing of proteins. Secondly, viral DNAs synthesis and assembly of capsids occur in the nucleus. In the case of some herpesvirus, it has been claimed that the virus maybe de-enveloped and re-enveloped through the cytoplasm. Thirdly, production of infectious progeny virus is invariably accompanied by the irreversible destruction of the infected cell. Lastly, the herpesvirus examined to date are able to remain latent in their natural hosts. In cells harboring latent virus, the viral genomes is in the form of closed circular molecules, and only a small subset of viral gene is expressed [1].

HSV-1 and HSV-2 were among the first viruses targeted by pharmaceutical companies because their diseases are easily diagnosed and are relatively widespread in developed countries. Acyclovir (ACV) heralded the second generation of antivirals for herpesviruses. As an orally available agent with low toxicity, it has become widely used for the treatment of primary and recurrent HSV-1 and HSV-2, the suppression of recurrent HSV, and the treatment of uncomplicated VZV [2]. However, ACV-resistant HSV strains were first reported in 1982 with most being recovered from immunocompromised patients previously treated with ACV [3,4]. The clinical virologists are

interested in antiviral of plant extracts since the effective life span of any antiviral drug is limited, viral diseases remain intractable to most of the orthodox antiviral drugs and the problem of viral resistance, latency and recurrence in immunocompromised hosts [5].

It has been estimated that the vascular plants in Thailand include at least 10,000 species of about 1,763 genera from 245 families. Thailand is endowed with a great diversity indigenous medicinal plant species. The Thais have a long tradition of using medicinal herbs and plants in folklore medicine but many of the claimed curative properties have not been scientifically proven [6]. Medicinal plants in Thailand were reported for anti HSV-1 and HSV-2 as *Eugenia caryophyllus* (Spreng.) Bullock & S.G.Harrison, *Inula cappa* (Ham. Ex D. Don) DC., *Cissus repanda* Vahl and *Drymaria diandra* Blume and *Houttuynia cordata* Thunb. [7, 8, 9, 10, 11].

Durian is a native plant of Brunei, Indonesia and Malaysia which has been known in the Western world for about 600 years. There are more than 30 recognised *Durio* species, at least nine of them are edible. *Durio zibethinus* is the only species well known in the international market while the other species are sold in their local markets. There are hundreds of durian cultivars but only 5 cultivars are found in Thailand such as *D. graveolens*, *D. griffithii*, *D. lowianus*, *D. mansonii* and *D. zibethinus* [12].

Seeds and the shells of durians are discarded as agricultural wastes. Most research work has focused on the flavor, phenolic contents and other nutritional properties of the edible portion of durian, and very rare research was concerned with the systematic characterization of bioactive secondary metabolites in durian seeds or seed coat, although some reports mentioned its use by local people to treat sores and wounds. Recently, it has been reported that durian seed contains a specific content of oligomeric proanthocyanidins [13]. Thus, it is interesting to determine HSV-2 inhibition by durian seed coat crude extracts for preliminary bioactive compounds

Materials and Methods

Plant material. Native seed durian collected from Lab-Lae District, Uttaradit Province during April-June 2015. Seed coat was peeled off from native seed durian and dried.

Plant extracts. Dried durian seed coat, 200 g, were milled and soaked with distilled water or ethanol by maceration method at room temperature for 3 days. Then, each solution was filtered and solvent was evaporated, using a rotary evaporator and dried with lyophilizer. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) before investigation of HSV-2 inhibition.

Cell Line and Virus. Vero cells was used in this study and grow in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated bovine calf serum (Hyclone) in a humidified 5% CO₂ incubator at 37 °C. Herpes simplex virus type 2 was used throughout the study. Quantitation of the virus was performed in 24-well tissue culture plates using a plaque titration assay.

Cytotoxicity test. Cytotoxicity test was performed following published procedures [14]. Each extract was diluted with MEM by serial 2-fold dilution and then adjusted cell line to 1×10^6 cells/ml. After that, added 100 µl of each extract concentration in 96-well tissue culture plate and added 100 µl of cell line in the same well. Then, incubated this plate in a humidified 5% CO₂ incubator at 37 °C. After 72 hours incubation, the cell were stained with 0.1% crystal violet in 1% ethanol. Finally, calculated the 50% cytotoxicity dose (CD₅₀)

Plaque reduction assay. The Vero cells were grown in 24-well tissue culture plates as a monolayer. Approximately 100 PFU of HSV were added to the cells per well and incubate at room temperature for 1 hour. After that, crude extract at non-toxic concentrations and acyclovir (ACV) at ED₅₀ concentration were applied into duplicate test wells whereas media were added into control wells. Growth media containing 2% sodium carboxyl cellulose were added to the Vero cells. After 2-3 days of incubation in the CO₂ incubator, the cells were stained with 0.1% crystal violet in 1% ethanol. The plaques were counted and inhibitory activities of test extracts were determined comparing to control. The 50% effective dose (ED₅₀) was calculated.

Results and Discussion

Dried native durian seed coat were milled (Fig.1) and extracted using distilled water and ethanol. Then, solvent was evaporated, using a rotary evaporator and dried with lyophilizer. Percentage of yield of each crude extract were shown in Table 1.



Fig.1 Dried native durian seed coat after milling

Table 1 Crude extracts obtained from native durian seed coat

Solvents	yield of crude extract (%)
Distilled water	3.85
Ethanol	1.30

Cytotoxicity test was performed. The native durian seed coat extracted with distilled water showed the CD_{50} of 224.40 $\mu\text{g/ml}$ whereas the native durian seed coat extracted with ethanol showed the lower CD_{50} of 112.47 $\mu\text{g/ml}$ (Table 2).

Table 2 The 50% Cytotoxicity doses (CD_{50}) of native durian seed coat crude extracts

Native durian seed coat crude extract	CD_{50} ($\mu\text{g/ml}$)
Aqueous extract	224.40
Ethanol extract	112.47

Studies on antiviral activities of medicinal plants have been performed using an *in vitro* experiment. Among various assay methods, plaque assay has always been used for detecting antiviral activities of both synthetic and natural products [15]. For the anti-HSV type 2 activities by the native durian seed coat crude extracts, the virus were not removed after incubation with Vero cells at room temperature for 1 hour. Ethanolic extract of durian seed coat showed the percentage of inhibition at various concentration (25, 50 and 100 $\mu\text{g/ml}$) higher than the aqueous extract of durian seed coat (Table 3). The crude extracts at the concentration of 100 $\mu\text{g/ml}$ displayed the percentage of HSV-2 inhibition at 36.34 ± 1.47 and 61.56 ± 5.30 for the aqueous extract (Fig.2) and ethanolic extract (Fig.3), respectively. Interestingly, plaque size after HSV-2 infection were reduced with the extracts compared with HSV-2 control. It revealed that these extracts might reflect a result of virus replication within the original infected cells and the virus progenies infecting neighboring cells. Thus, when the plaque sizes were smaller than controls as observed in extract treated wells, it was possible to conclude that the extracts affected either the expansion of the infectious foci or interfered with the production of infectious virions [7].

Table 3 HSV-2 inhibition by durian seed coat crude extracts

Native durian seed coat crude extract	Concentration ($\mu\text{g/ml}$)	HSV-2 inhibition (%)
Aqueous extract	25.00	12.01 ± 7.01
	50.00	18.32 ± 0.66
	100.00	36.34 ± 1.47
Ethanollic extract	25.00	23.01 ± 0.06
	50.00	31.53 ± 5.92
	100.00	61.56 ± 5.30

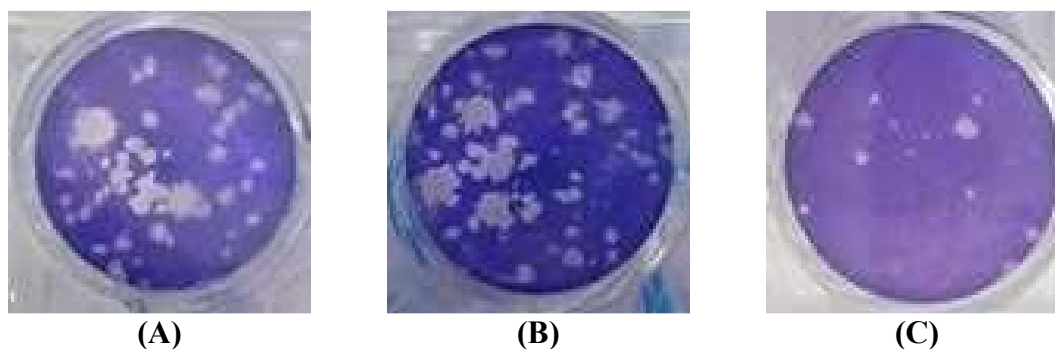


Fig.2 HSV-2 inhibition by native durian seed coat extracted with distilled water at concentration of $100 \mu\text{g/ml}$ (A), HSV-2 control (B), and acyclovir at ED_{50} concentration ($9.30 \mu\text{g/ml}$) (C)

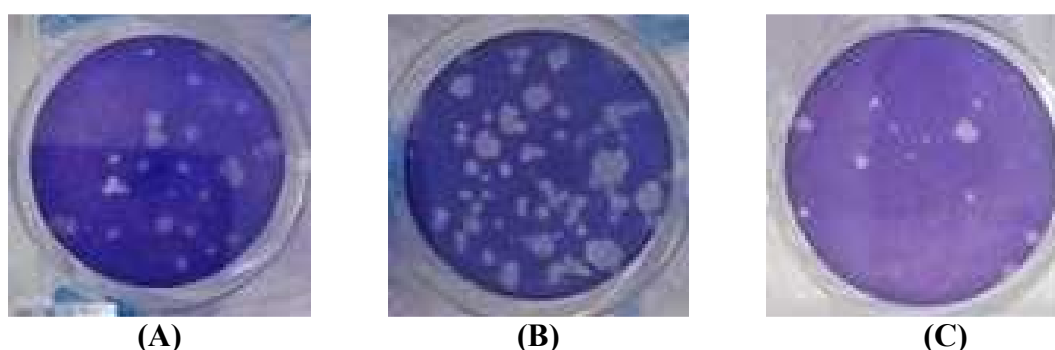


Fig.3 HSV-2 inhibition by native durian seed coat extracted with ethanol at concentration of $100 \mu\text{g/ml}$ (A), HSV-2 control (B), and acyclovir at ED_{50} concentration ($9.30 \mu\text{g/ml}$) (C)

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