

Anti herpes simplex virus property of *Phyllanthus urinaria* (L) as shown by plaque reduction assay

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Abstrak

Obat tradisional telah populer di Indonesia. Banyak di antara obat tersebut yang efektif untuk menyembuhkan pelbagai penyakit, termasuk penyakit infeksi. Berdasarkan hal tersebut, aktifitas anti Herpes Simplex Virus ekstrak *Phyllanthus urinaria* (L) diteliti secara *in vitro* dengan metoda "plaque reduction assay". Hasil menunjukkan bahwa ID₁₀₀ dicapai oleh ekstrak pada konsentrasi 6% dan 4% v/v untuk HSV-2 dan HSV-1

Abstract

Traditional medicine has been popular in Indonesia. Many of them are effective for curing various diseases. Based on that, antiviral activity to Herpes Simplex Virus of an aqueous phase of extract of *Phyllanthus urinaria* (L) plant was investigated *in vitro* by a plaque reduction assay. Result showed that ID₁₀₀ was achieved by the extract at concentration of 6% and 4% v/v for HSV-2 and HSV-1 respectively.

Keywords : *Phyllanthus urinaria* (L)-Herpes Simplex Virus-traditional medicine-plaque reduction assay

INTRODUCTION

Indonesian peoples has long been using traditional medication in daily life. Many traditional medicine derived from plants or herbs proved to be effective for curing various diseases, including diabetes, hypertension, renal stone and some infectious diseases.

At last 145 different plants have been documented and reported to have pharmacological properties.¹ Even with the introduction of modern medicine the practice of using traditional medication is still continuing. Furthermore, several centers for research and development of traditional medicine have been established, such as "Pusat Pengembangan Obat Tradisional" at Gadjah Mada University.

Phyllanthus urinaria (L), or "daun gendong anak" been used effectively for treatment of hepatitis, urinary tract infection, bacillary dysentery, ureteral colic, toothache, and as diuretic.^{1,2} Since the most common cause of hepatitis is viral origin, we predicted that extract of this plants may have antiviral activity. However, because of difficulties in isolation of hepatitis

viruses or even they cannot be adapted in cell culture system, we used herpes simplex virus to evaluate *in vitro* antiviral activity of extract of *Phyllanthus urinaria* (L).

METHODS

Preparation of the extract

One gram of whole plant was cut into small pieces and finely ground. This material was transferred into conical tube of 15 ml and added with 9 ml of aquadest and mixed well by brief vortexing. The material was left over night at room temperature to allow water soluble substance to dissolve. On the next day the mixture was spinned down, supernatant collected and filtered through a membrane of 0,45 µm pore size. This supernatant containing water soluble extract of the plant is used for the experiment.

Evaluation of antiviral activity by reduction assay method³

Vero cell line was cultured in Dullbeco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotic in tissue culture flasks. Culture was incubated at 37°C in a humidified incubator with 5% CO₂ atmosphere. After confluent

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monolayer growth was achieved, medium was replaced with 2 ml of fresh DMEM containing 5% FCS. 0,2 ml suspension of 103 plaque forming unit/ml of HSV 1 and HSV 2 were added into individual flasks and the cultures returned into incubator. After 2 hours incubation, flasks were taken out from the incubator and the medium decanted. The culture were rinsed with phosphate-buffered saline to remove any remaining virus particles, after which 5 ml of DMEM supplemented with 5% FCS was added into the flasks. 400 µl, 300 µl, 200 µl, 100 µl, 50 µl, of the extract were added to individual flasks. Two flasks of culture infected with HSV 1 and HSV 2, respectively were not treated with the extract and served as controls. One flasks of uninfected culture was used as negative control. These procedures were done in duplicate.

All cultures were reincubated at 37°C for three days. At the end of incubation period medium was decanted and the cultures was stained with crystal violet stain to visualised the plaques. Number of plaques on each flasks was compared to that of control.

Interpretation of the result

Antiviral activity of the extract is expressed of plaque formation and calculated by the formula :

$$\frac{\text{No. of plaque of control} - \text{No. of treated culture}}{\text{No. of plaque of control}} \times 100\%$$

The amount of extract capable of reducing plaque number by 100% is said to be inhibitory dose 100(ID₁₀₀).

RESULT

Total inhibition of plaque formation (ID₁₀₀) was achieved by the extract with the concentration of 6% v/v for HSV-2 and 4% for HSV-1, respectively (table 1).

Concentration of 0,5% v/v has no effect on inhibition of plaque formation on HSV-1 and HSV-2 infected Vero cell was enhanced by addition of the extract. This growth enhancement was clearly seen before and after staining procedure; extract – treated cultures were more deeply stained than those of controls (see figure1).

DISCUSSION

Our study showed that extract of *Phyllanthus urinaria* (L) has in vitro activity against Herpes Simplex Virus. Inhibitory Dose₁₀₀ was achieved by the extract at concentration of 6% v/v and 4% v/v for HSV-2 and HSV-1, respectively. It means that ID₁₀₀ will be 60 µl/ml and 40 µl/ml. Since we used crude extract in this study, we estimated much lower concentration is required to get ID₁₀₀ if we used purified extract in the assay.

Table 1. Inhibitory effect of *Phyllanthus urinaria* (L) extract on plaque formation of HSV-1 and HSV-2 infected Vero cell line

Addition of extract (µl)	Final concentration of extract (%v/v)	* Number of Plaque on cultures infected with		% inhibition of plaque formation	
		HSV-1	HSV-2	HSV-1	HSV-2
400	8	0	0	100	100
300	6	0	0	100	100
200	4	0	36	100	53,80
100	2	31	42	50,70	46,14
50	1	52	55	17,40	29,60
25	0,5	67	82	0	0
0 (control)	0	63	78	-	-

* Mean

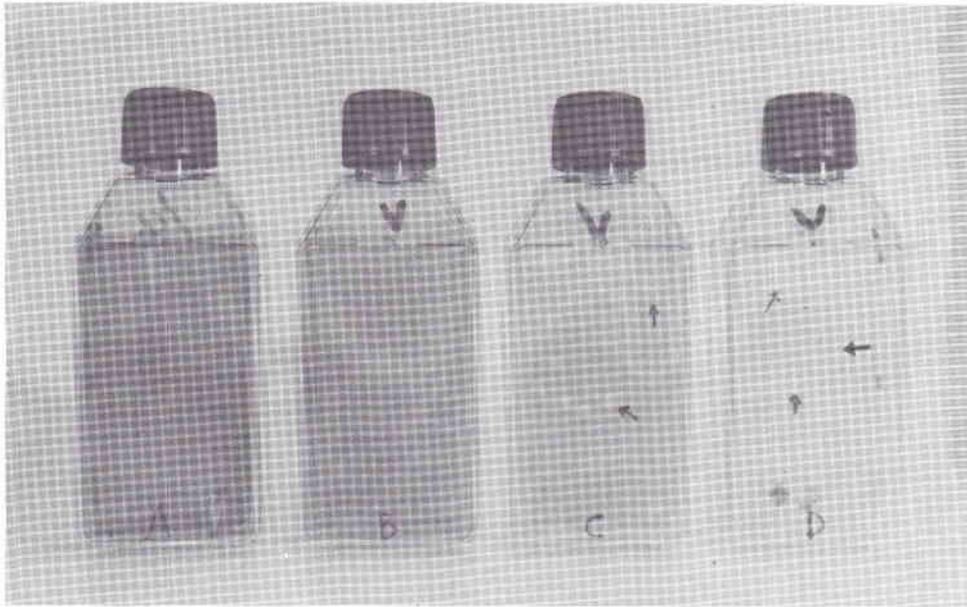


Figure 1. HSV-infected Vero cell line. A, B and C: cultures treated with extract of *Phyllanthus urinaria* (L). D: untreated culture (control). Arrows indicate positions of the plaques. Note that treated are more densely stained than control.

Enhancement of the growth of Vero cell line in extract treated cultures indicate that there was no toxic effect of this extract on the cell. It is very likely that the extract contains high concentration of vitamins and minerals which promote growth of the cells.

Specific treatment of HSV infection is usually not required unless serious complication involving visceral organs are anticipated. This is, in part because of relatively expensive commercially available synthetic antiviral drugs for most people in Indonesia. Unfortunately, untreated cases of HSV infection will be potential sources of spreading the virus to other people. In addition, several studies showed that some strains of HSV were resistance to antiviral drugs.^{4,5} The problems were also complicated by the presence of asymptomatic cases who shed the virus persistently and by the ability of the disease to become latent.^{6,7} Despite possible involvement of the brain in HSV infection, especially in immune compromised patients, more attention should be paid to HSV infection during pregnancy as it may cause spontaneous abortion, stillbirth, and congenital malformation or, more frequently, mucocutaneous infection occurring at the time of delivery.⁸

In order to overcome part of these problem, we have been searching back for anti HSV agent derived from

plants readily available in our environment. Our previous study showed that another plants, *Momordica charantia* or spring cucumber, also has *in vitro* activity against HSV-2.⁹ *In vivo* activity of these extract are now under investigation. Further studies are also needed to elucidate other pharmacological aspects, including mechanism of action and possible effect of the extract on other viruses. The search for active substance having antiviral effect from these plants is also important. Nevertheless, from practical point of view, it will be more beneficial if the whole plant or the crude extract can be used as an alternative of anti HSV agents as it will much facilitate its use in treatment of HSV infection.

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