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Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection in vitro and in vivo

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Abstract

Twenty Thai medicinal plant extracts were evaluated for anti-herpes simplex virus type 1 (HSV-1) activity. Eleven of them inhibited plaque formation of HSV-1 more than 50% at 100 μg/ml in a plaque reduction assay. *Aglaia odorata, Moringa oleifera*, and *Ventilago denticulata* among the 11 were also effective against thymidine kinase-deficient HSV-1 and phosphonoacetate-resistant HSV-1 strains. These therapeutic efficacies were characterized using a cutaneous HSV-1 infection in mice. The extract of *M. oleifera* at a dose of 750 mg/kg per day significantly delayed the development of skin lesions, prolonged the mean survival times and reduced the mortality of HSV-1 infected mice as compared with 2% DMSO in distilled water ($P < 0.05$). The extracts of *A. odorata* and *V. denticulata* were also significantly effective in limiting the development of skin lesions ($P < 0.05$). There were no significant difference between acyclovir and these three plant extracts in the delay of the development of skin lesions and no significant difference between acyclovir and *M. oleifera* in mean survival times. Toxicity of these plant extracts were not observed in treated mice. Thus, these three plant extracts may be possible candidates of anti-HSV-1 agents.

Keywords: Antiviral agent; Herpes simplex virus; Therapeutic efficacy; Thai medicinal plant

1. Introduction

Herpes simplex virus type 1 (HSV-1) causes labial herpes, keratitis, and encephalitis. The herpetic infection is common to humans and a major cause of morbidity especially in immunosuppressed patients. Acyclovir (ACV) and other nucleoside derivatives, penciclovir, famciclovir, valaciclovir, and ganciclovir have been approved for treatment of herpes simplex virus (HSV-1 and HSV-2) infections worldwide (Galasso et al., 1997; Leung and Sacks, 2000). Screening of plant extracts for an anti-herpetic activity has given interesting results for the search for new antiviral agents (Berghe et al., 1993; Nagasaka et al., 1995; Namba et al., 1997; Kurokawa et al., 1998; Kurokawa et al., 1999; Garcia et al., 1999; Lin et al., 1999; Xu et al., 1999; Ikeda et al., 2000).

In many developing countries, traditional medicine is still the mainstay of health care, and most of the drugs and cures used come from plants. Traditional medicines were exploited for use in pharmaceutical products for new and effective remedies, or for use in treating many common and some uncommon conditions. The rising financial burden of pharmaceutical products in Thailand, and importing them, has been a strong added incentive to integrate traditional medicine into the overall health care provision in Thailand. At present there are about 60 types of medicinal plants that have already been promoted to use in primary health care and classified according to their pharmacological actions such as treatment of peptic ulcers, anti-flatulence, laxative, anti-diarrheal and anti-herpetic, etc. The extract of *Clinacanthus nutans* has been traditionally used in Thailand for the topical treatment of herpes simplex virus and varicella–zoster virus infections (Sangkitporn et al., 1993; Sangkitporn et al., 1995). The anti-HSV activities in vitro of some Thai medicinal plant extracts, *Aglaia edulis*, *Centella asiatica*, *Glyptopetalum sclerocarpum*, *Maclura*

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cochinchinensis, and *Mangifera indica*, have been reported (Saifah et al., 1999; Sotanaphun et al., 1999; Yoosook et al., 2000). There are many potential Thai medicinal plants to be investigated on the scientific basis and evaluated as the candidates for anti-HSV agents. The development of Thai traditional medicine and application in health care is supported and included in the plan of Ministry of Public Health of Thailand.

This project aims to investigate for the anti-HSV-1 activity of Thai medicinal plants.

2. Materials and methods

2.1. Viruses and cells

HSV-1 strains used were wild-type 7401H HSV-1 (Kurokawa et al., 1993), thymidine kinase-deficient (TK^-) HSV-1 (B2006) (Dubbs and Kit, 1964), and phosphonoacetate-resistant (AP^r) HSV-1 (Kurokawa et al., 1995). The other viruses used were poliovirus type 1 (Sabin strain) and measles virus (Tanabe strain). Virus stocks were prepared from infected-cultured cells as reported previously (Shiraki and Rapp, 1988; Kurokawa et al., 1993). Vero cells were grown and maintained in Eagle's minimum medium (MEM) supplemented with 5 and 2% calf serum, respectively.

2.2. ACV

ACV was purchased as tablets from Nippon Wellcome K.K. A tablet (200 mg) was powdered in distilled water.

2.3. Preparation of plant extracts

Plant specimens were collected from Mae Wong, Kamphaengphet., in May 2001 and the medicinal plant garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, in April 2002. The plants were identified by Dr. R. Suttisri (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University). Voucher specimens of these plants were deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Water and ethanol extracts were prepared from fresh plants listed in Table 1. Briefly, for water extract, 250 g of fresh plant was boiled in 500 ml of distilled water for 1 h. The aqueous extract was filtered, concentrated, and lyophilized. For ethanol extracts, 250 g of fresh plant was extracted in 500 ml of ethanol for 24 h, and the extract was filtered and dried. Some ethanol extracts were further extracted in chloroform, and the extracts were dried.

In a plaque reduction assay, stock solution of 10 mg/ml in dimethylsulfoxide (DMSO) for solvent extract and in sterile distilled water for water extract were prepared. In mouse infection model, the extracts were prepared at 20 mg/ml in 2% DMSO solution, as a stock solution.

2.4. Plaque reduction assay

Vero cells, in 60 mm tissue culture dishes, were infected with 100 plaque forming units (PFU)/0.2 ml of wild-type HSV-1, TK^- HSV-1, AP^r HSV-1, poliovirus, or measles virus. After 1 h incubation at room temperature for virus

Table 1
List of plants used in this study

Plants	Family	Part use[a]	Solvent[b]
Acacia pennata L. Willd.	Leguminosae	Leaf	Water
Aglaia odorata Lour.	Meliaceae	Leaf	Ethanol
Azadirachta indica A. Juss.	Meliaceae	Leaf	Water
Bischofia javanica Blume	Bischofiaceae	Leaf	Ethanol
Cassia siamea Lam.	Leguminosae	Leaf	Ethanol
Cerbera odollam Lam.	Apocynaceae	Leaf	Water
Clausena excavata Burm. f.	Rutaceae	Leaf	Chloroform
Elaeocarpus grandiflorus Sm.	Elaecarpaceae	Leaf	Ethanol
Garcinia cowa Roxb.ex DC.	Guttiferae	Leaf	Chloroform
Harpullia arborea (Blanco) Radlk.	Sapindaceae	Leaf	Ethanol
Hura crepitans L.	Euphorbiaceae	Leaf	Ethanol
Ipomoea aquatica Forsk.	Convolvulaceae	Leaf	Ethanol
Mangifera indica L.	Moringaceae	Leaf	Water
Moringa oleifera Lam.	Moringaceae	Leaf	Ethanol
Protium serratum Engl.	Burseraceae	Leaf	Ethanol
Rinorea anguifera (Lour) Kuntze	Violaceae	Leaf	Ethanol
Schima wallichi (DC.) Korth.	Theaceae	Leaf	Ethanol
Strychnos minor Dennst.	Strychnaceae	Leaf	Ethanol
Ventilago denticulata Willd.	Rhamnaceae	Leaf	Ethanol
Willughbeia edulis Roxb.	Apocynaceae	Leaf	Chloroform

[a] The leaf was used for extraction for all plants according to the primarily screening test for the antiviral activity.
[b] Most extracts were ethanol extract except some were water extract and chloroform extract according to the traditional use and the activity of the partitioned fraction in solvent.

adsorption, the cells were overlaid with 5 ml of 0.8% nutrient methylcellulose containing 100 μg/ml of the plant extract. The infected cultures were incubated for 2, 3, and 5 days at 37 °C for poliovirus, HSV-1, and measles, respectively. The infected cells were fixed and stained and then the number of plaques was counted (Shiraki et al., 1991; Kurokawa et al., 1993). The effective concentrations for 50% plaque reduction (EC_{50}) were determined from the curve relating the plaque number to the concentration of the samples.

The cytotoxicity of the plant extracts was evaluated by detachment of the cells from the surface of stained dishes with methylene blue in the plaque formation method (visible cytotoxicity) (Kurokawa et al., 1993). Cytotoxicity was represented as follows: (−), no detachment of cells from the surface of dish; (+/−), thick coloration of cell layer and <10% of cell detachment; (+), 10–50% of cell detachment; (++), >50% of cell detachment; and (+++), complete detachment. Cytotoxicity was also examined by the growth inhibition of Vero cells (Kurokawa et al., 1995). Briefly, Vero cells were seeded at a concentration of 1.5×10^5 cells in 60 mm dish and grown at 37 °C for 2 days. The culture medium was replaced by fresh medium containing each of plant extracts at various concentrations, and cells were further grown for 2 days. The cells were treated with trypsin and the number of viable cells was determined by the trypan blue exclusion method. The concentrations of plant extracts reducing cell viability by 50% (CC_{50}) were determined.

2.5. Efficacy of plant extracts in a cutaneous mouse HSV-1 infection model

Female BALB/c mice (6 weeks old) were purchased from Sankyo Labs Service Co. Ltd., Tokyo, Japan. The right midflank of each mouse was chipped and depilated with a chemical depilatory, hair remover. One or two days later, the naked skin was scratched using a 27-guage needle and 5 μl of HSV-1 (7401H strain) suspension of 1×10^6 PFU was applied to the scarified area (Kumano et al., 1987; Kurokawa et al., 1993). Plant extract (250 mg/kg per dose) or ACV (5 mg/kg per dose) was orally administered by using a gavage at 8 h before and three times daily for at least 14 days after HSV-1 infection. The development of skin lesions and mortality was continuously observed three times daily and scored as follows: 0, no lesion; 2, vesicles in local region; 4, erosion and/or ulceration in local region; 6, mild zosteriform lesion; 8, moderate zosteriform lesion; and 10, severe zosteriform and death. The infected mice were held at least for 20 days after infection. The toxicity of plant extracts was assessed in infected mice by the loss of body weight compared with the control group. The mice were weighed on 1–7, 10, and 15 days. We conducted procedures in conforming to the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Experimentation Guidelines of the Toyama Medical and Pharmaceutical University.

2.6. Statistical analysis

The Student's *t*-test was used to evaluate the significant differences between control and plant extract-treated mice in mean times at which score 2 or 6 or death was initially observed after infection. The repeated measure ANOVA was used to analyze the interaction between plant extract-treated mice and control mice in mean skin lesions for 3–10 days after infection. Statistical differences in the mortality were evaluated using the Chi-square test. A *P*-value of less than 0.05 was defined as statistically significant.

3. Results

3.1. Effects of plant extracts on plaque formation

To evaluate the antiviral activities of 20 medicinal plant extracts (Table 1), the inhibitory effects on the plaque formation of wild-type HSV-1, poliovirus, or measles virus (Table 2) were examined. The plant extracts that inhibited plaque formation more than 50% were selected as virus inhibitory plant extracts at a concentration of 100 μg/ml. Eleven plant extracts exhibited anti-HSV-1 activities as indicated in Table 2. Four plant extracts, *Aglaia odorata*, *Cerbera odollam*, *Harpullia arborea*, and *Ventilago denticulata* inhibited plaque formation of all three viruses. *Azadirachta indica* and *Protium serratum* were effective against HSV-1 and poliovirus. *Rinorea anguifera* was effective against HSV-1 and measles virus and *Elaeocarpus grandiflorus* were effective against poliovirus and measles virus. *Hura crepitans*, *Moringa oleifera*, *Schima wallichi*, and *Willughbeia edulis* inhibited specifically plaque formation of HSV-1. *Bischofia javanica* was specifically inhibitory to poliovirus. *Cassia siamea*, *Clausena excavata*, and *Strychnos minor* specifically inhibited measles virus. Most of the plant extracts tested were not cytotoxic except for *C. siamea*, *C. excavata*, and *E. grandiflorus* that showed less than 50% cytotoxicity. The therapeutic indexes (CC_{50}/EC_{50}) of *A. odorata*, *M. oleifera* and *V. denticulata* were 32.9, 8.8, and 18.1, respectively (Table 2).

3.2. Activity of plant extract against wild-type and drug resistant HSV-1 strains

Antiviral activities of *A odorata*, *M. oleifera*, and *V. denticulata* against HSV-1 TK^-, AP^r, and wild-type 7401H strains were examined to evaluate their modes of anti-HSV-1 action. As shown in Table 3, the EC_{50} values of ACV for TK^- and AP^r strains were much greater than that of 7401H strain. *Moringa oleifera* was more effective to AP^r strain and *A. odorata* was less effective to TK^- strain than that of the wild-type strain. The susceptibility of ACV-resistant strains to *V. denticulata* was similar to that of the wild-type strain.

Table 2
Antiviral assay of plant extracts by a plaque reduction assay

Plant extracts	Plaques percent of control[a] (100 μg/ml)			HSV-1/EC_{50}[b] (μg/ml)	Cytotoxicity[c] (100 μg/ml)	CC_{50}[d] (μg/ml)	CC_{50}/EC_{50}
	HSV-1	Polio	Measles				
Acacia pennata	98.3	62.5	100		−		
Aglaia odorata	0[e]	0[e]	0[e]	9.5 ± 0.7	−	312 ± 17	32.9
Azadirachta indica	46.3[e]	16.7[e]	100		−		
Bischofia javanica	97	37.5[e]	78.9		−		
Cassia siamea	93.6	100	49.7[e]		+		
Cerbera odollam	0[e]	0[e]	0[e]	0.4 ± 0.1	−		
Clausena excavata	57.1	100	15.3[e]		+		
Elaeocarpus grandiflorus	76.3	16.7[e]	28.7[e]		+		
Garcinia cowa	58.2	100	100		−		
Harpullia arborea	19.9[e]	45.8[e]	32.9[e]		−		
Hura crepitans	46.6[e]	100	100		−		
Ipomoea aquatic	86.4	84	100		−		
Mangifera indica	69.7	100	100		−		
Moringa oleifera	49.5[e]	100	100	100.0 ± 5.3	−	875 ± 35	8.8
Protium serratum	23.8[e]	37.5[e]	100		−		
Rinorea anguifera	43.1[e]	100	9.6[e]		−		
Schima wallichi	33.1[e]	100	71.3		−		
Strychnos minor	76.5	100	41.1[e]		−		
Ventilago denticulata	0[e]	0[e]	0[e]	46.3 ± 1.5	−	838 ± 53	18.1
Willughbeia edulis	100	51.5	88.0 ± 2.0	–	−		
ACV				0.2 ± 0.1			

[a] Percent plaque formation compared with control without plant extract. Values are mean of duplicate dishes in an experiment.
[b] Mean ± S.D. of three independent experiments.
[c] +++, 100% cytotoxic; ++, >50% cytotoxic; +, 10–50% cytotoxic; +/−, <10% cytotoxic; −, not cytotoxic observed by visible cytotoxicity as described in text.
[d] 50% cell cytotoxicity (CC_{50}) determined by trypan blue exclusion method. Mean ± S.D. of three independent experiments.
[e] Plant extracts that reduced plaque formation of HSV-1 more than 50%.

3.3. Efficacy of plant extracts in a cutaneous mouse HSV-1 infection model

Among the plant extracts that inhibited plaque formation of HSV-1, five of them, *A. odorata*, *C. odollam*, *M. oleifera*, *V. denticulata*, and *W. edulis* were selected for preliminary examination in a cutaneous HSV-1 infection model in mice. The plant extracts were orally administered to mice (five mice per group) every 8 h for 5 days after infection at the dose of 250 mg/kg per dose, corresponding to the doses for other traditional herb used in human. Three of them (*A. odorata*, *M. oleifera*, and *V. denticulata*) significantly delayed the development and progression of skin lesions and/or prolonged mean survival times ($P < 0.05$ by Student's *t*-test, data not shown) as compared with control (2% DMSO in distilled water treatment). Then anti-HSV-1 therapeutic efficacy of *A. odorata*, *M. oleifera*, and *V. denticulata* was further analyzed. The infected mice received ACV or extracts for 14 consecutive days via oral gavage. *Moringa oleifera* at 250 mg/kg per dose delayed the development and progression of skin lesions of score 2 and score 6, prolonged mean survival times ($P < 0.05$ by Student's *t*-test), and reduced the mortality of HSV-1 infected mice ($P < 0.05$ by Chi-square test) as compared with control (Table 4 and Fig. 1). *Aglaia odorata* and *V. denticulata* significantly delayed the development and progression of skin lesions of score 2 ($P < 0.05$ by Student's *t*-test). There were no significant difference in reduction of mortality of infected mice in the group treated with *A. odorata* and *V. denticulata* compared to the control group ($P = 0.07$ and 0.66, respectively, analyzed by Chi-square test). There was no significant difference between ACV and these three plant extracts in the delay of the development of skin lesions and no significant difference in mean survival times between ACV and *M. oleifera*. When the plant extracts were examined for toxicity in infected mice, the mouse weights among groups were similar on days 7 and 14 (data not shown), indicating no toxicity.

Table 3
Activity of plant extract against wild-type and drug resistance HSV-1 strains

Plant extracts	EC_{50} (μg/ml)[a]		
	7401H strain	TK^- strain	AP^r strain
ACV	0.2 ± 0.1	>40	>40
Aglaia odorata	9.5 ± 0.7	24.5 ± 3.2	10.8 ± 1.8
Moringa oleifera	100.0 ± 5.3	130.0 ± 7.1	43.5 ± 2.1
Ventilago denticulata	46.3 ± 1.5	52.0 ± 4.2	42.0 ± 2.8

[a] Effective concentration for 50% plaque reduction; EC_{50} values were determined from three independent experiments.

Table 4
Effects of plant extracts on cutaneous HSV-1 infection in BALB/c mice

Plant extracts	Dose[a] (mg/kg per dose)	Mean time[b] (days ± S.D.)			Mortality[c]
		Score 2	Score 6	Survival	
Control		4.0 ± 0	6.3 ± 1.3	7.4 ± 0.8	10/10
Acyclovir	5	5.3 ± 1.6[d]	7.3 ± 1.0[d]	13.4 ± 0.8[d]	1/10[f]
Aglaia odorata	250	7.3 ± 0.8[d]	6.9 ± 1.9	8.6 ± 2.6	9/10
Moringa oleifera	250	5.6 ± 1.2[d]	7.8 ± 0.4[d]	12.0 ± 2.6[d]	6/10[f]
Ventilago denticulata	250	4.8 ± 1.0[d]	6.6 ± 1.4	8.6 ± 4.0	8/9

[a] Plant extract was orally administered at 8 h before and three times daily for at least 14 days after HSV-1 infection.
[b] Mean time at which score 2 or 6 was first observed after infection.
[c] Number of dead mice/number of mice tested, observed at 14 days after HSV-1 infection.
[d] Mean time was significantly prolonged ($P < 0.05$ vs. control by repeated analysis).
[f] Mortality was significantly reduced ($P < 0.05$ vs. control by Chi-square test).

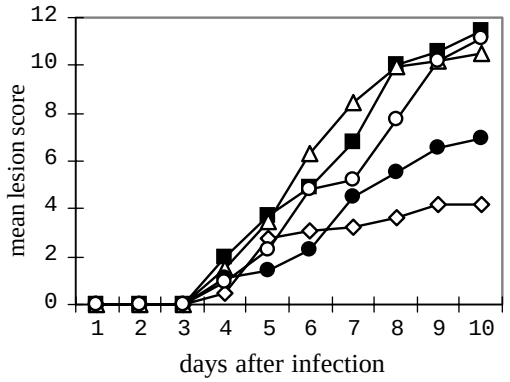

Fig. 1. Effect of plant extracts on mice infected with HSV-1. Ten mice in each group were infected, ACV (5 mg/kg per dose) or plant extracts (250 mg/kg per dose) were orally administered three times daily for 14 days after infection, (■), 2% DMSO in distilled water; (◇), ACV; (△), *A. odorata*; (●), *M. oleifera*; (○), *V. denticulata* (ACV and *M. oleifera* were significantly effective ($P < 0.05$) against control, by the repeated measures ANOVA). These results are part of the same experiment as that of Table 4.

4. Discussion

Aglaia odorata, *M. oleifera*, and *V. denticulata* were found to be effective in cutaneous HSV-1 infection in mice (Table 4). Many Thai medicinal plants showing anti-HSV activity were reported by Sotanaphun et al. (1999), Yoosuk et al. (2000), Phrutivorapongkul et al. (2002), and Akanitapichat et al. (2002). However, those were only in vitro activities and the therapeutic antiviral efficacies of plant extracts in vivo have not been characterized. There is no report of anti-HSV activities for these three plant extracts. *Moringa oleifera* and *A. odorata* are traditional herbs used in Southeast Asia and Indo-China. Every part of these plants is of value for food and used for stimulation of digestion and treating septicemia (Verma et al., 1976; Perry, 1980) and for remedy of venereal and of diseases of the chest, as stimulant, antipyretic, tonic, and as treatment of convulsions (Perry, 1980). The biological activity of *V. denticulata* has not been reported. Therefore, our results are the first evidence demonstrating that the extracts of *A. odorata*, *M. oleifera*, and *V. denticulata* exhibited therapeutic antiviral efficacy in vivo.

In a plaque reduction assay, some of plant extracts with anti-HSV-1 activity also exhibited anti-poliovirus activity and/or anti-measles virus activity. Since these viruses have different structure and different replication cycles, their differences in sensitivity to the various plant extracts may be due to the different modes of antiviral action of the active compounds in the plant extracts.

AP^r HSV-1 strain was more susceptible to *M. oleifera* and TK$^-$ HSV-1 strain was less susceptible to *A. odorata*. The susceptibility of these viruses to *V. denticulata* was similar to that of wild HSV-1 strain (Table 3). All these three plant extracts showed similar antiviral activity against HSV-2 (Baylor 186) compared to activity against HSV-1 (7401H) (data not shown). Since the growth of HSV-1 resistant strains to ACV and/or phosphonoacetate was inhibited by the plant extracts, modes of anti-HSV-1 action of plant extracts were different from those of ACV or phosphonoacetate. Thus, these three plant extracts can be used as anti-HSV agents and the possible additive or synergistic anti-HSV activity may be expected in combined use with ACV or phosphonoacetate.

Moringa oleifera delayed the development and progression of skin lesion and reduced the mortality of infected mice (Table 4). This plant extract exhibited anti-HSV-1 activity in vitro but did not exhibit anti-poliovirus or anti-measles virus activity (Table 2). Therefore, it is suggested that this plant extract showed therapeutic efficacy for HSV-1 infection in vivo possibly based on its anti-HSV-1 specific activity observed in vitro. In addition, EC_{50} value of *M. oleifera* was 100 μg/ml and higher than those of *A. odorata* and *V. denticulata*. However *M. oleifera* significantly reduced the mortality of infected mice, although *A. odorata* and *V. denticulata* did not (Table 4). The protective effect of *M. oleifera* in mice was not related to a direct antiviral effect. This may partly result from an immunomodulatory activity and/or higher bioavailability of *M. oleifera*. *Aglaia odorata* and *V. denticulata* also exhibited therapeutic anti-HSV activity in mice. Thus, these three plant extracts might contain the active compounds that would be effective in treatment of the cutaneous HSV-1 infection. The treatment

with higher dose of these plant extracts than 750 mg/kg per day may show more effective therapeutic anti-HSV-1 efficacy in mice. These results also suggested that antiviral components in the plant extracts were selectively absorbed from alimentary tracts and elicited an HSV-1 inhibitory effect which was not associated with toxicity. Thus, these plant extracts may be evaluated to be possible candidates of new plant extracts for anti-herpes simplex virus infection.

The utilization of medicinal plant extracts as drugs or medicines or supplements based on the biological activities from the scientific basis provides the system for use in health care services. Therefore, the use of these medicinal plant extracts, especially *M. oleifera*, may be applicable as prophylactic or therapeutic anti-HSV medicines for the treatment of HSV-1 infection and may be effective against ACV-resistant variants that may occur during ACV therapy. The mechanism of action, the toxicity in non-infected mice and clinical study of these three plant extracts will be further investigated.

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References

Akanitapichat, P., Kurokawa, M., Tewtrakil, S., Pramyothin, P., Sripandkulchai, B., Shiraki, K., Hattori, M., 2002. Inibitory activities of Thai medicinal plants against herpes simplex type 1, poliovirus type 1, and measles virus. J. Tradit. Med. 19, 174–180.

Berghe, V.D.A.R., Haemers, A., Vlietinck, A.J., 1993. Antiviral agents from higher plants and an example of structure-activity relationship of 3-methoxy flavones. In: Colgate, S.M., Molyneux, R.J. (Eds.), Bioactive Natural Product: Detection, Isolation and Structural Determination. CRC Press, Boca Raton, pp. 405–440.

Dubbs, D.R., Kit, S., 1964. Mutant strains of herpes simplex virus deficient in thymidine kinase-inducing activity. Virology 22, 493–502.

Galasso, G.J., Whitley, R.J., Merigan, T.C., 1997. Antiviral Agents and Human Viral Diseases. Lippincott-Raven, Philadelphia.

Garcia, G., Cavallaro, L., Broussalis, A., Ferraro, G., Martino, V., Campos, R., 1999. Biological and chemical characterization of the fraction with antiherpetic activity from *Achyrocline flaccida*. Planta Med. 65, 343–346.

Ikeda, T., Ando, J., Miyazono, A., Zhu, X.H., Tsumagari, H., Nohara, T., Yokomizo, K., Uyeda, M., 2000. Anti-herpes virus activity of *Solanum* steroidal glycosides. Biol. Pharm. Bull. 23, 163–164.

Kumano, Y., Yamamoto, M., Mori, R., 1987. Protection against herpes simplex virus infection in mice by recombinant murine interferon-β in combination with antibody. Antivir. Res. 22, 175–188.

Kurokawa, M., Ochiai, H., Kazuhiko, N., Neki, M., Xu, H., Kadota, S., Sutardjo, S., Matsumoto, T., Namba, T., Shiraki, K., 1993. Antiviral traditional medicines against herpes simplex virus (HSV-1), poliovirus, and measles virus and their therapeutic efficacies for HSV-1 infection in mice. Antivir. Res. 22, 175–188.

Kurokawa, M., Nagasaka, K., Hirabayashi, T., Uyama, S., Sato, H., Kagayama, T., Kadota, S., Ohyama, H., Hozumi, T., Namba, T., Shiraki, K., 1995. Efficacy of traditional herbal medicine in combination with acyclovir against herpes simplex virus type 1 infection in vitro and in vivo. Antivir. Res. 27, 29–37.

Kurokawa, M., Hozumi, T., Basnet, P., Nakano, M., Kadota, S., Namba, T., Kawana, T., Shiraki, K., 1998. Purification and characterization of eugeniin as an anti-herpes virus compound from *Geum japonicum* and *Syzygium aromaticum*. J. Pharmacol. Exp. Ther. 284, 728–735.

Kurokawa, M., Basnet, P., Ohsugi, M., Hozumi, T., Kadota, S., Namba, T., Kawana, T., Shiraki, K., 1999. Anti-herpes simplex virus activity of moronic acid purified from *Rhus javanica* in vitro and in vivo. J. Pharmacol. Exp. Ther. 289, 72–78.

Leung, D.T., Sacks, S.L., 2000. Current recommendations for the treatment of genital herpes. Drugs 60, 1329–1352.

Lin, Y.M., Flavin, M.T., Sahure, R., Chen, F.C., Sidwell, R., Barnard, D.L., Huffman, I.H., Kein, E.R., 1999. Biflavonoids isolated from *Rhus succedanea* and *Garcinea multiflora* exhibited moderate anti-HSV-1, and HSV-2 activities. Planta Med. 65, 120–125.

Nagasaka, K., Kurokawa, M., Imakita, M., Terasawa, K., Shiraki, K., 1995. Efficacy of Kakkon-to, a traditional herb medicine, in herpes simplex virus type 1 infection in mice. J. Med. Virol. 46, 28–34.

Namba, T., Shiraki, K., Kurokawa, M., 1997. Development of antiviral agents from traditional medicines. Towards natural medicine research in the 21st century. In: Proceedings of the International Symposium on Natural Medicines, October 28–30, Kyoto, Japan.

Perry, L.M., 1980. Medicinal Plants of East and Southeast Asia. MIT Press, Cambridge, MA, p. 260.

Phrutivorapongkul, A., Lipipun, V., Ruangrungsi, N., Watanabe, T., Ishikawa, T., 2002. Studies on the constituents of seeds of *Pachyrrhizus erosus* and their anti-herpes simplex virus (HSV) activities. Chem. Pharm. Bull. 50, 534–537.

Saifah, E., Suttisri, R., Shamsub, S., Pengsuparp, T., Lipipun, V., 1999. Bisamides from *Aglaia edulis*. Phytochemistry 52, 1085–1088.

Sangkitporn, S., Polihan, K., Thawatsupa, P., Bunchok, M., Chawalitthumrong, P., 1993. Treatment of recurrent genital herpes simplex virus infections with *Clinacanthus nutans* extract. Bull. Dept. Med. Serv. 18, 226–231.

Sangkitporn, S., Chaiwat, S., Balachandra, K., Dechotiwongse Na-Ayudhaya, T., Bunchok, M., Jawasu, C., 1995. Treatment of herpes zoster with *Clinacanthus nutans* (BiPhya Yaw) extract. J. Med. Assoc. Thai. 78, 624–627.

Shiraki, K., Rapp, F., 1988. Effects of caffeine on herpes simplex virus. Intervirology 29, 235–240.

Shiraki, K., Ishibashi, M., Okuno, T., Namazue, J., Yamanishi, K., Sonoda, T., Takahashi, M., 1991. Immunosuppressive dose of azathioprine inhibits replication of human cytomegalovirus in vitro. Arch. Virol. 117, 165–171.

Sotanaphun, U., Lipipun, V., Suttisri, R., Bavovada, R., 1999. A new antiviral and antimicrobial sesquiterpene from *Glyptopetalum sclerocarpum*. Planta Med. 65, 257–258.

Verma, S.C., Banerji, R., Misra, G., Nigam, S.K., 1976. Nutritional value of Moringa. Curr. Sci. 45, 769–770.

Xu, H.X., Lee, S., Lee, S.F., White, R.L., Blay, J., 1999. Isolation and characterization of an anti-HSV polysaccharide from *Prunella vulgaris*. Antivir. Res. 44, 43–54.

Yoosook, C., Bunyapraphatsara, N., Boonyakiat, Y., Kantasuk, C., 2000. Antiherpes simplex virus activities of crude water extracts of Thai medicinal plants. Phytomedicines 6, 411–419.