

*Full Length Research Paper*

# Inhibitory effect of aromatic herbs, lavender, sage and chamomile against herpes simplex virus infection

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**This study demonstrated anti-herpes simplex virus (HSV) activity of lavender, sage and chamomile extracts. Green monkey kidney cells were protected from HSV-2 infection by dichloromethane and methanol extract of lavender with therapeutic indices (TI) of 1.98 and 2.90, respectively when the cells were treated before viral infection. Moreover, the dichloromethane extract of chamomile showed the highest TI which was 20.74 against HSV-1 when the virus was treated during adsorption. The inhibitory effect of dichloromethane extract of lavender significantly had the highest TI (45.90) when HSV-1 was treated after viral adsorption. The highest inhibitory effect against HSV-2 was observed after treatment of the virus with methanol and dichloromethane extracts of sage, with TIs of 9.02 and 10.1, respectively. Chamomile extracts showed significant virucidal effects on both HSV-1 and HSV-2 viral particles. The inhibitory effect of chamomile extracts on HSV-1 and HSV-2 viral yield after viral replication was the highest at 30 h after treatment. Respective reductions of viral titers by 9.7 and 7.1 fold were observed when cells were treated with dichloromethane and methanol extract of chamomile. Therefore, chamomile, sage and lavender were demonstrated to possess anti-herpes simplex virus activities at various stages of the viral multiplication cycle.**

Key words: Chamomile, sage, lavender, herpes simplex virus (HSV), anti-herpes simplex virus.

## INTRODUCTION

Herpes simplex virus (HSV) is endemic in all human populations. There are two closely relative types of HSV. HSV-1 is the primary agent of orolabial disease or fever blisters. The primary symptoms of herpes simplex virus infection include a prodromal flu-like syndrome with fever, headache, malaise, and diffuse myalgias, followed by local symptoms consisting of itching and appearance of painful papules (Khan et al., 2005). Recurrent herpes labialis is the most frequent clinical manifestation of reactivated HSV-1 infection (Astani et al., 2011). HSV remains latent in sensory neurons after primary infection. Recurrent infection is characterized by episodic

reactivation with milder symptoms compared to primary infection (Astani et al., 2011; Khan et al., 2005; Wagner et al., 2008). HSV reactivation is generated by stress, radiation, and other related factors such as sunlight, menstruation and therapeutic irradiation (Collier and Oxford, 2000; Wagner et al., 2008). Genital herpes involves HSV-2 and is an important sexually transmitted disease, contributing to risk of HIV infection. After primary infection, the virus ascends peripheral sensory nerves and becomes established in sensory or autonomic nerve root ganglia, evading immune attack (Brugha et al., 1997). Recurrent genital disease is due to reactivation of the initial strain of virus from recently infected sacral nerve root ganglia (Erlich et al., 1989; Hill et al., 2009; Severson and Tyring, 1999; Yoosook et al., 2000).

Since viruses are intracellular parasites and utilize organelles within host cells, it is difficult to completely eliminate a virus. The clinical manifestations of HSV associated disease exhibit different severity in immunocompetent patients. However, in immunocompromised

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**Abbreviations:** HSV, Herpes simplex virus; TI, therapeutic indices.

patients and neonates, herpetic infections can cause serious systemic illnesses. Antiviral agents licensed currently for treatment of HSV infections include acyclovir (ACV) and derivatives. Some such antiviral agents may produce toxic side-effects. ACV is most commonly used for the treatment of HSV infection, being the first of the nucleoside analogues that are chain-terminating inhibitors highly specific for HSV-infected cells. It has been used successfully in both topical and internal applications with both HSV-1 and HSV-2. However, ACV is expensive, and a major problem associated with ACV therapy is the development of drug-resistant strains of HSV. Mutations in the HSV-thymidine kinase or DNA polymerase genes of HSV may occur after long-term treatment and these mutants are particularly important as opportunistic infectious agents in immunocompromised patients (Crumpacker et al., 1982; Elion, 1993).

Antiviral agents from medicinal plants with new effective compounds exhibiting different modes of action against viral infections are urgently needed. Plants of the Lamiaceae family, including lavender and sage, and of the Compositae family, including chamomile, are widely used in traditional medicines. They have been shown to possess biological properties such as insecticidal, fungicidal and anti-tumorigenic activities (Carta et al., 1996; Ho et al., 2000; Li, 1998; Maria et al., 1998; Moshe et al., 1993). Therefore, the aim of the present study was to investigate possible anti-HSV activity of sage, lavender and chamomile extracts. Moreover, the mode of action of the extracts on HSV multiplication cycle was also studied.

## MATERIALS AND METHODS

### Cell lines and viruses

Green monkey kidney (GMK) cells were obtained from Assist. Prof. Dr. Weerah Wongkham, Department of Biology, Chiang Mai University, Thailand. GMK cells were grown in monolayers with Eagle's minimum essential medium (MEM) (Hyclone, UK) supplement with 10% heat inactivated fetal calf serum (Starrate, Australia) and 40 µg/ml gentamycin. Cells were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> incubator. HSV-1 strain F and HSV-2 strain G were propagated and grown on GMK cells. The virus stock was prepared from supernatants of infected cells and stored at -85°C until use. The viral infectivity titers were determined by plaque titration assay on confluent GMK cells and were expressed as plaque forming units (PFU) per ml.

### Plant extracts and acyclovir

Chamomile, sage and lavender were purchased from the Thai Royal Project. Dried plant materials were cut into small pieces, ground, and soaked in dichloromethane and methanol at room temperature. The ratio of plant material to solvent is 50 g per 5 L. The extracts were filtered, concentrated and lyophilized to form dried extracts. The dried extracts were reconstituted in dimethylsulfoxide (DMSO) to be a stock concentration of 160 mg/ml and further diluted in medium for the determination of cytotoxicity and of anti-HSV activity.

Acyclovir, a commonly used anti-HSV synthetic drug, was used

as a positive control (ACV, Sigma Aldrich). ACV was dissolved in sterile distilled water and was diluted with MEM before determination of anti-HSV activity. 50% inhibition concentration (IC<sub>50</sub>) of ACV was calculated.

### Cytotoxicity assay

For the cytotoxicity assay, the effects of all extracts on the GMK cells were determined in 96-well tissue culture plates. Reconstituted extracts were diluted two-fold by MEM and added onto the cell culture plate. Final concentrations of DMSO in plant preparations after dilution were less than 1% v/v, which did not affect the cells. Then, GMK cells at  $1 \times 10^6$  cells/ml were seeded into the culture plate and incubated for 4 days. The cells were stained with 0.1% crystal violet in 1% ethanol for 15 minutes. The cytotoxic concentration of the extract that reduced viable cell number by 50% (CD<sub>50</sub>) was determined from dose-response curves and calculated according to modified protocol of Reed and Muench (1938). Thus, non-toxic concentrations of dichloromethane extract of chamomile used in this study ranged from 1 to 156 µg/ml while dichloromethane extracts of lavender and sage ranged from 1 to 9 µg/ml. Methanol extract of chamomile showed nontoxic concentrations, which ranged from 2 to 313 µg/ml whereas methanol extracts of lavender and sage ranged from 2 to 19 µg/ml, which were used throughout the study.

### Plaque titration assay

GMK cells were seeded into 24-well tissue culture plates and incubated at 37°C in a 5% CO<sub>2</sub> incubator. Viral stocks were serially diluted in MEM and each dilution was added to the cell monolayer. After 1 h adsorption, the infected cells were then overlaid with overlay medium containing 1.5% carboxymethylcellulose and incubated at 37°C in a 5% CO<sub>2</sub> incubator for 4 days before staining with 0.1% crystal violet in 1% ethanol for 15 min. Virus plaques were counted and expressed as plaque forming units (PFU) per ml.

### Effect of plant extracts on pretreated cells

Cell monolayers were treated for 1 h with non toxic concentrations of plant extracts as mentioned in cytotoxicity assay. The extracts were removed before adding HSV inoculum. After incubation of the cells with HSV at room temperature for 1 h, overlay medium was added. The infected cells were incubated at 37°C in a 5% CO<sub>2</sub> incubator for 4 days. The number of plaques was counted and compared with controls.

### Effect of plant extracts on HSV during viral adsorption

Confluent cell monolayers cultivated in 24-well tissue culture plate were infected with 200 PFU of HSV. Then, non toxic concentrations of plant extracts as mentioned in cytotoxicity assay were added onto cell monolayers and incubated for 1 h at room temperature for virus adsorption. After viral adsorption, the residual inoculum was removed and replaced by overlay medium containing 1.5% carboxymethylcellulose, and incubated at 37°C in a 5% CO<sub>2</sub> incubator for 4 days. After incubation, the virus plaques were stained with 0.1% crystal violet in 1% ethanol for 15 min. The number of plaques was counted and the 50% effective doses (ED<sub>50</sub>) were determined from dose-response curves and expressed as 50% inhibition of plaque numbers compared with controls.

### Effect of plant extracts on HSV after viral adsorption

Confluent cell monolayers cultivated in 24-well tissue culture plates were infected with 200 PFU of HSV and incubated for 1 hour at room temperature for virus adsorption. After viral adsorption, non toxic concentrations of the plant extracts as mentioned in cytotoxicity assay were added onto the infected cells. The cells were overlaid with overlay medium and incubated for 4 days at 37°C in a 5% CO<sub>2</sub> incubator. The number of plaques was counted and the 50% effective doses (ED<sub>50</sub>) were determined from dose-response curves and expressed as 50% inhibition of plaque numbers compared with controls.

### Effect of plant extracts on viral replication

Cells were grown in 25 cm<sup>2</sup> flasks until confluence, after which, the cells were infected with 1 × 10<sup>6</sup> PFU/ml of HSV. After incubation, the infected cells were washed twice with PBS and treated with the highest non toxic concentrations of the plant extract and IC<sub>50</sub> of ACV as positive control. Dichloromethane extracts of lavender, sage and chamomile were used at concentrations of 9, 9 and 156 µg/ml, respectively whereas the methanol extracts of lavender, sage and chamomile were used at concentrations of 19, 19 and 313 µg/ml, respectively. Virus-infected cells in flasks containing medium with 2% fetal calf serum were also included as negative controls. The infected cells were further incubated at 37°C in a 5% CO<sub>2</sub> incubator and the cells were collected at 18, 24 and 30 h after viral infection. The infected cells were frozen and thawed twice before determination of virus titers using a plaque titration assay.

### Direct inactivation of viral particles

For investigation of the effects of plant extracts on viral particles, the highest non-toxic concentrations of each extract were mixed with HSV and incubated for 1, 2, 3 and 4 h at room temperature. Dichloromethane extracts of lavender, sage and chamomile were used at concentrations of 9, 9 and 156 µg/ml, respectively whereas the methanol extracts of lavender, sage and chamomile were used at concentrations of 19, 19 and 313 µg/ml, respectively. After incubation, the inactivated viral particles were adsorbed onto cells for 1 h at room temperature. The infected cells were overlaid with overlay medium containing 1.5% carboxymethylcellulose and incubated for 4 days at 37°C. Then, infected cells were stained with 0.1% crystal violet. The percentages of viral plaque inhibition by the extracts were calculated and compared with those of untreated controls.

### Statistical analysis

Data were given as mean ± S.D of three independent experiments. Statistical comparison between groups was analyzed by one way analysis of variance (ANOVA) and Post hoc Tukey test. The *p* values less than 0.05 (*p* < 0.05) were considered significance.

## RESULTS

Both dichloromethane and methanol extracts were tested for toxicity to GMK cells using the method of Reed and Muench (1938). The cytotoxicity assay yielded CD<sub>50</sub> values of 13.77, 13.77 and 158.87 µg/ml for dichloromethane extracts of lavender, sage and chamomile, respectively whereas the CD<sub>50</sub> values for methanol

extracts of lavender, sage, and chamomile were 19.97, 19.97 and 316.98 µg/ml respectively.

At non toxic concentrations, reconstituted extracts were tested against both standard HSV-1 and HSV-2. In order to determine the mode of antiviral action of plant extracts, the potential inhibitory effects were tested on pretreated cells, during and after viral adsorption using a plaque reduction assay. After pretreatment of the cells with the extracts, only dichloromethane and methanol extracts of lavender were effective on HSV-2, with respective ED<sub>50</sub> values of 6.95 and 6.87 µg/ml, and therapeutic indices (TI; CD<sub>50</sub>/ED<sub>50</sub>) of 1.98 and 2.90 whereas the percentage of HSV-1 inhibition was 40 and 35% after treatment with the highest concentration of dichloromethane and methanol extracts of lavender at concentrations of 13.77 and 19.91 µg/ml, respectively. Moreover, other extracts inhibited the virus less than 50% and thus ED<sub>50</sub> values could not be calculated (Table 1).

The methanol extract of lavender showed the highest TI of 11.95 followed by the methanol extract of chamomile (TI of 4.70) during HSV-1 adsorption to the cells. Dichloromethane extract of chamomile showed inhibitory effects on HSV-1 during adsorption to cells with the highest TI of 20.74 followed by dichloromethane extracts of lavender and sage with TIs of 14.2 and 1.94, respectively. The methanol extract of sage and dichloromethane extract of lavender possessed the highest anti-viral activities during HSV-2 adsorption with TI values of 4.81 and 2.44, respectively (Table 1).

The dichloromethane extract of lavender on HSV-1 after viral adsorption showed significantly the highest TI of 45.90 whereas the significantly highest inhibitory effects against HSV-2 were observed after treatment of the virus with methanol and dichloromethane extract of sage, with TIs of 9.02 and 10.1, respectively.

Direct inactivation of HSV-1 and HSV-2 by the plant extracts was evaluated by a plaque titration assay at 1, 2, 3 and 4 h after viral inactivation, and results compared with the untreated virus control. Chamomile extracts showed significantly highest virucidal effects on both HSV-1 and HSV-2 particles. HSV-1 viral particles were completely inactivated within 3 h, whereas HSV-2 viral particles were completely inactivated by dichloromethane extract of chamomile within 1 h. Moreover, HSV-2 viral particles were also completely inactivated by methanol extract of chamomile within 2 h of treatment (Table 2).

Replication of both standard HSV-1 and HSV-2 after treatment with the extracts was compared with untreated control viruses at 0, 18, 24 and 30. ACV at IC<sub>50</sub> concentration was used as positive control, which was 1.5 and 1.3 µg/ml for HSV-1 and HSV-2 treatment, respectively. At 30 h incubation, the cytopathic effect of HSV-1 and HSV-2 infected cells control was observed by 90%, and log HSV-1 and HSV-2 titers (PFU/ml) were 11.5 ± 0.04 and 9.70 ± 0.29, respectively.

HSV-1 viral yield was reduced after treatment with the extracts within 30 h. The highest reduction in log HSV-1 titer was 7.06 after treatment with methanol extract of

**Table 1.** Inhibitory effects of HSV by dichloromethane and methanol extracts of lavender, chamomile and sage on pretreated cells, during and after viral adsorption.

Test		ED <sub>50</sub> (µg/ml)					
		Pretreated cell		During viral adsorption		After viral adsorption	
		HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2
<b>Methanol extract</b>							
Lavender	LM	-	6.87±0.23 (2.90±0.09) <sup>A</sup>	1.67±0.52 (11.95±4.72) <sup>B,C</sup>	5.03±0.05 (3.97±0.04) <sup>D</sup>	2.61±0.07 (7.64±0.19) <sup>A</sup>	3.77±0.07 (5.29±0.09) <sup>B</sup>
		-	-	-	4.15±0.03 (4.81±0.04) <sup>E</sup>	6.10±0.06 (3.27±0.03) <sup>A</sup>	2.22±0.15 (9.02±0.59) <sup>C</sup>
Chamomile	CM	-	-	68.02±8.27 (4.70±0.55) <sup>A,B</sup>	111.38±4.91 (2.85±0.12) <sup>C</sup>	104.84±1.39 (3.02±0.03) <sup>A</sup>	112.78±3.32 (2.81±0.08) <sup>A</sup>
<b>Dichloromethane extract</b>							
Lavender	LD	-	6.95±0.07 (1.98±0.02) <sup>B</sup>	0.97±0.43 (14.2±6.7) <sup>C</sup>	5.65±0.07 (2.44±0.03) <sup>B</sup>	0.30±0.03 (45.90±4.64) <sup>B</sup>	2.34±0.06 (5.88±0.14) <sup>B</sup>
		-	-	7.09±0.11 (1.94±0.03) <sup>A</sup>	6.39±0.04 (2.15±0.01) <sup>A</sup>	5.01±0.01 (2.75±0.01) <sup>A</sup>	1.36±0.20 (10.10±1.53) <sup>C</sup>
Chamomile	CD	-	-	7.66±1.46 (20.74±3.74) <sup>C</sup>	80.33±2.78 (1.98±0.07) <sup>A</sup>	65.02±1.34 (2.44±0.05) <sup>A</sup>	79.07±1.30 (2.01±0.03) <sup>A</sup>

( ) = (TI=CD<sub>50</sub>/ED<sub>50</sub>); Data in table are given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Tukey test. Values with the different alphabets within each column are significantly different ( $P < 0.05$ ).

chamomile, which was significantly higher than the reduction in log HSV-1 titer by 1.61 after treatment with ACV at IC<sub>50</sub> concentration. The reductions in log virus titer (PFU/ml) were 3.82, 3.62 and 4.87 after treatment with the dichloromethane extract of lavender, sage and chamomile, respectively. The reductions in log virus titer (PFU/ml) were 2.51 and 3.08 after treatment with the methanol extract of lavender and sage, respectively (Figures 1 and 2).

The highest reduction in log HSV-2 titer by 9.70 was demonstrated after treatment with dichloromethane extract of chamomile, which was significantly higher than the reduction in log HSV-2 titer by 3.46 after treatment with ACV at IC<sub>50</sub> concentration. Inhibition of HSV-2 yield at 30 h after viral infection was observed since log virus titers (PFU/ml) were reduced by 2.67 and 3.48 after treatment with the dichloromethane extracts of lavender and sage, respectively. The reductions in log virus titer (PFU/ml) of HSV-2 were 3.58, 4.08 and 4.48 after treatment with the methanol extract of lavender, sage and chamomile, respectively (Figures 3 and 4).

## DISCUSSION

Many aromatic plants used in phytotherapy are considered to be important sources for the production of raw

materials or preparations containing phytochemicals that have significant activity against microorganisms. In this study, the dichloromethane and methanol extracts of lavender were able to prevent pretreated cells from viral infection. Although, some extracts inhibited HSV by less than 50%, cytopathic effects of the infected cells after treatment with the extract were reduced and small plaque sizes were observed compared with the controls, which may have resulted from reduction of viral infectivity.

Other plants had been reported to affect the initial stages of viral infection before the adsorption period, for example the essential oil of *Mentha piperita* (Schuhmacher et al., 2003), chamomile oils (Koch et al., 2008) and *Phyllanthus urinaria* (Yang et al., 2005).

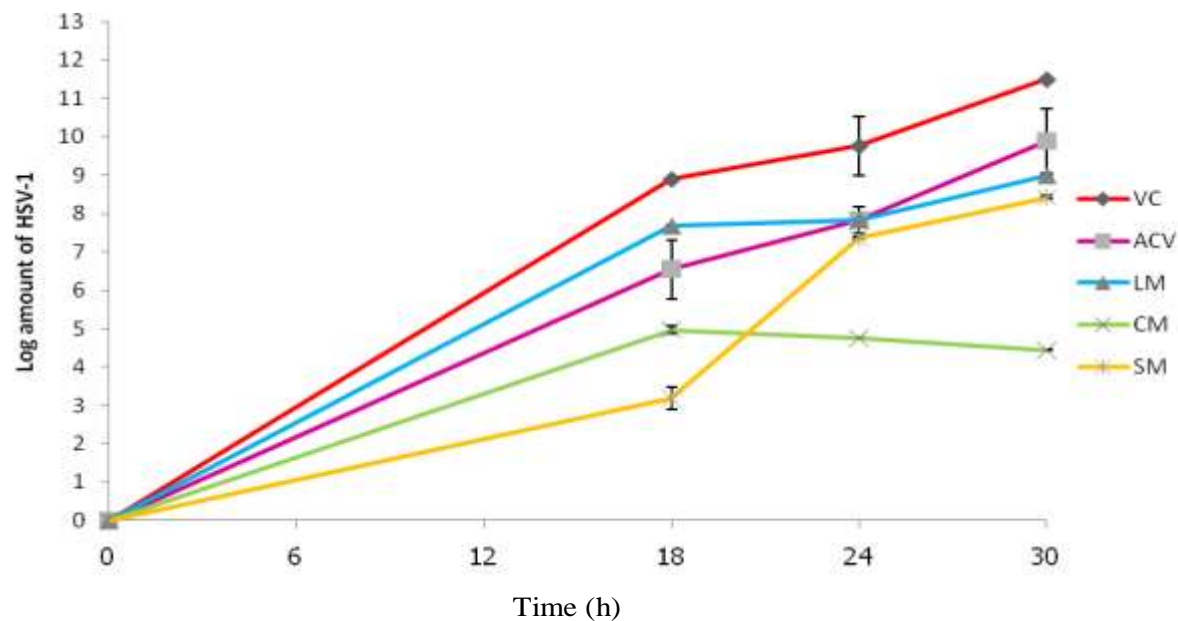
Here, the efficiency of the studied extracts was demonstrated on various stages of the virus replication cycle. The dichloromethane extract of chamomile was the most effective on HSV-1 adsorption due to the highest therapeutic index while the significantly highest activity on HSV-2 adsorption was observed when treating with the methanol extract of sage. However, the most effective extracts against HSV-1 and HSV-2 after adsorption were the dichloromethane extracts of lavender and sage, respectively.

Direct inactivation of both HSV-1 and HSV-2 was shown by a reduction of the amount of plaque by 100%, after treatment with the dichloromethane extract of

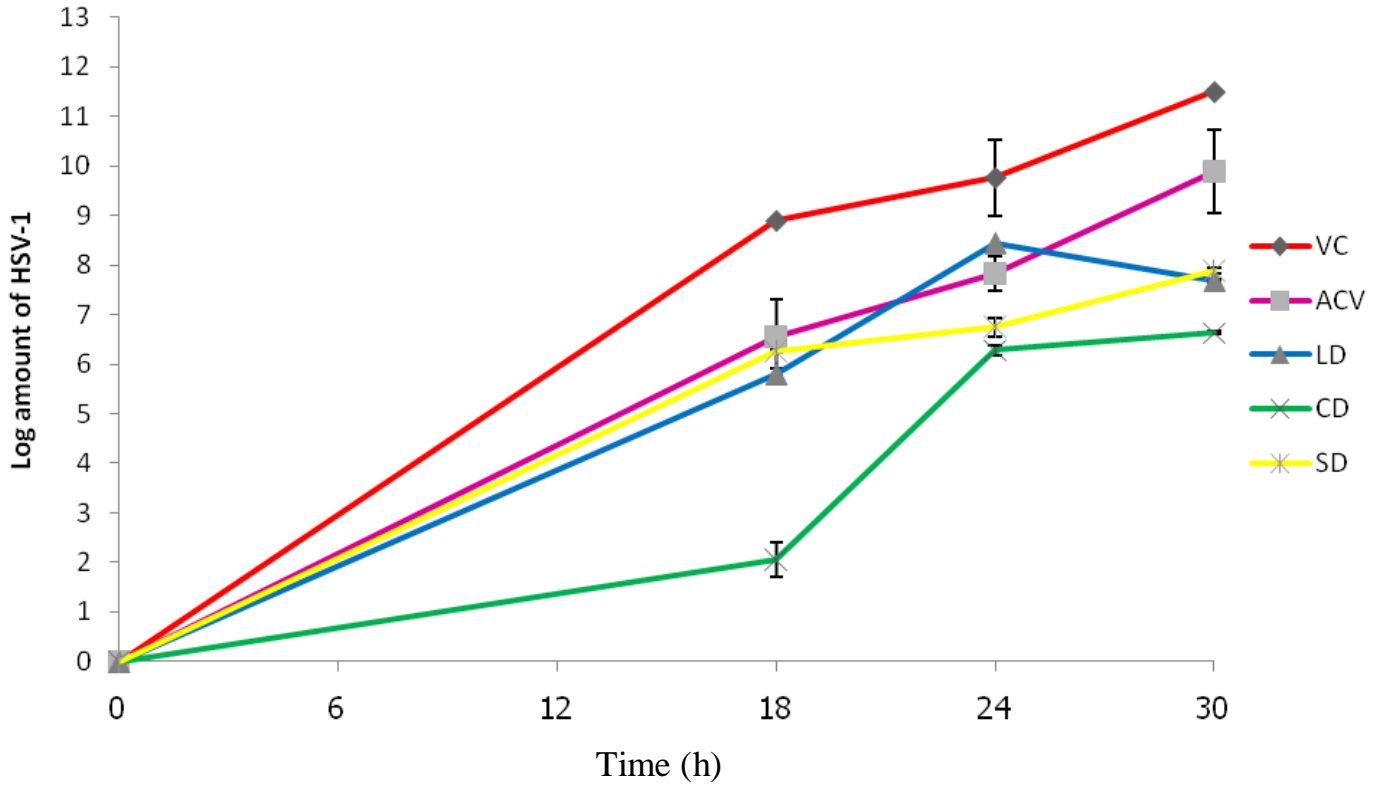
**Table 2.** Direct inactivation of HSV by dichloromethane and methanol extracts of lavender, chamomile and sage.

Test	Log amount of viruses (PFU/ml) at one hour interval								
	1		2		3		4		
	HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2	
<b>Methanol extract</b>									
Lavender	LM	5.29± 0.23 <sup>D</sup>	4.49± 1.20 <sup>D</sup>	5.37± 1.05 <sup>E</sup>	3.91± 0.19 <sup>C</sup>	5.3± 0.35 <sup>E</sup>	3.96 ±0.78 <sup>D</sup>	5.21± 0.30 <sup>D</sup>	3.88± 0.65 <sup>C,D</sup>
Sage	SM	4.87± 1.50 <sup>B,C</sup>	3.95± 1.60 <sup>C</sup>	4.78± 0.80 <sup>C</sup>	3.94± 0.90 <sup>C</sup>	4.81 ±0.80 <sup>C,D</sup>	3.28 ±0.70 <sup>C</sup>	4.63± 0.60 <sup>C</sup>	3.70 ±0.50 <sup>C</sup>
Chamomile	CM	4.76± 0.70 <sup>B</sup>	2.32 ±0.30 <sup>B</sup>	4.19± 0.60 <sup>B</sup>	<1 <sup>A</sup>	3.30±1.10 <sup>B</sup>	<1 <sup>A</sup>	3.00 ±0.46 <sup>B</sup>	<1 <sup>A</sup>
<b>Dichloromethane extract</b>									
Lavender	LD	5.09± 0.20 <sup>C,D</sup>	4.62± 0.9 <sup>D</sup>	5.11± 0.30 <sup>D</sup>	4.36 ±0.40 <sup>D</sup>	5.13 ±0.65 <sup>D,E</sup>	4.05 ±0.10 <sup>D</sup>	5.02 ±0.50 <sup>D</sup>	4.19 ±0.6 <sup>D</sup>
Sage	SD	4.85± 1.35 <sup>B,C</sup>	3.94± 0.35 <sup>C</sup>	3.93 ±0.10 <sup>A</sup>	2.81± 0.73 <sup>B</sup>	4.72 ±0.90 <sup>C</sup>	2.70 ±0.56 <sup>B</sup>	4.53 ±0.40 <sup>C</sup>	2.2 ±0.18 <sup>B</sup>
Chamomile	CD	4.23± 0.30 <sup>A</sup>	<1 <sup>A</sup>	4.13± 0.60 <sup>A,B</sup>	<1 <sup>A</sup>	<1 <sup>A</sup>	<1 <sup>A</sup>	<1 <sup>A</sup>	<1 <sup>A</sup>
Virus control		6.38 ±0.07 <sup>E</sup>	6.47± 1.20 <sup>E</sup>	6.12± 0.85 <sup>F</sup>	6.77± 0.20 <sup>E</sup>	6.18± 1.50 <sup>F</sup>	6.73± 0.60 <sup>E</sup>	6.3± 0.19 <sup>E</sup>	6.56± 0.80 <sup>E</sup>

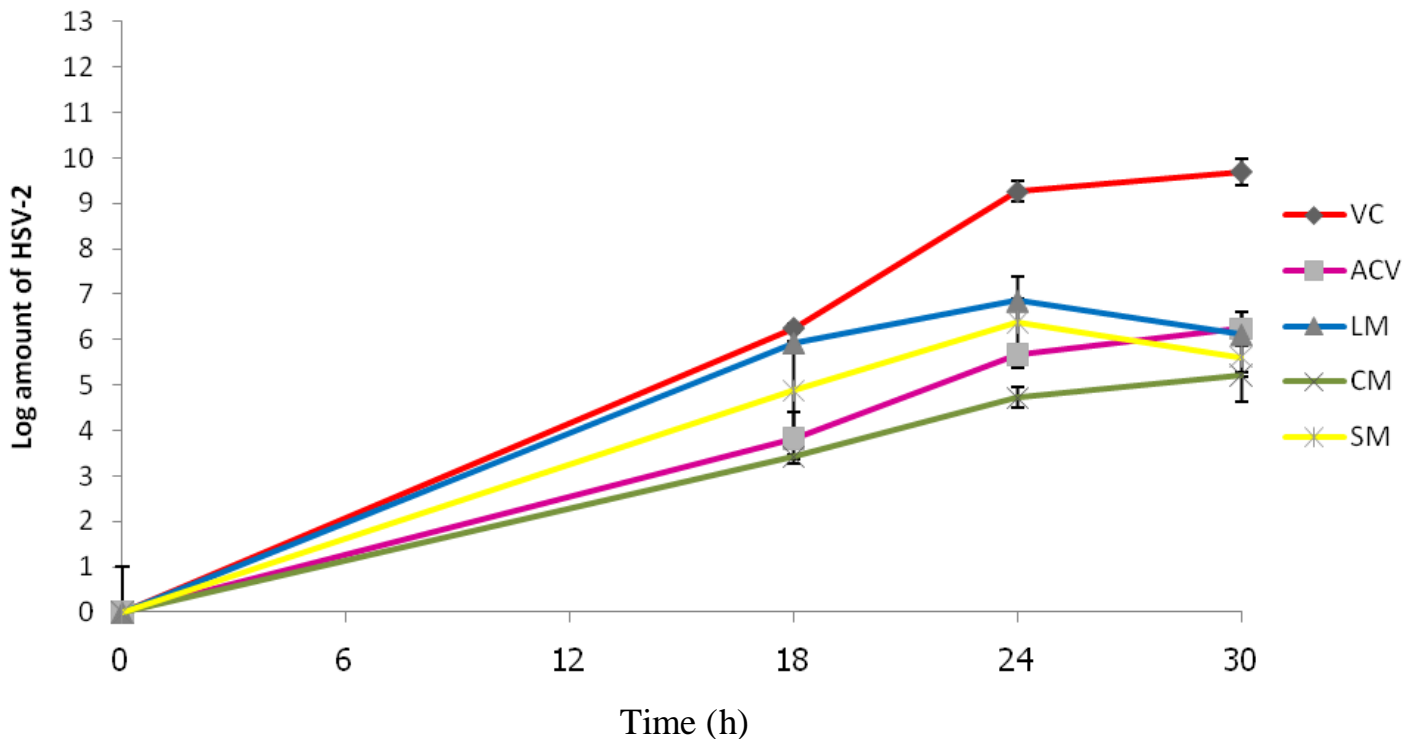
Data in table are given as mean ± standard deviation (SD) from triplicate experiments. Statistical comparison between groups applied using Post hoc Tukey test. Values with the different alphabets within each column are significantly different ( $P < 0.05$ ).



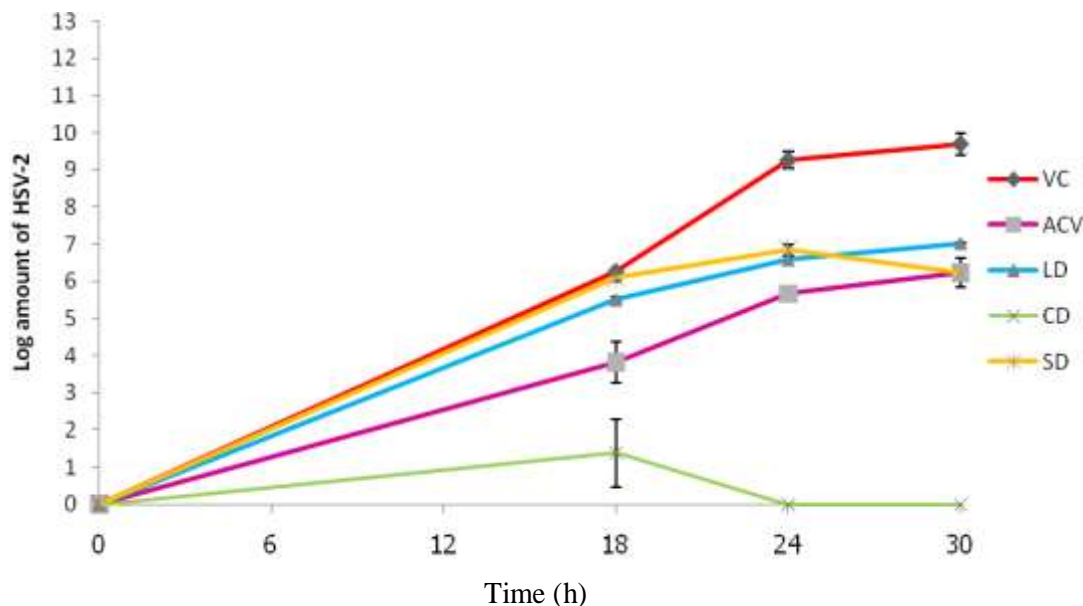
**Figure 1.** Titer of HSV-1 at 0, 18, 24 and 30 h after treatment with methanol extract of lavender (LM) 19 µg/ml, sage (SM) 19 µg/ml and chamomile (CM) 313 µg/ml compared with untreated virus control and ACV at ED<sub>50</sub> concentration



**Figure 2.** Titer of HSV-1 at 0, 18, 24 and 30 h after treatment with dichloromethane extract of lavender (LD) 9 µg/ml, sage (SD) 9 µg/ml and chamomile (CD) 156 µg/ml compared with untreated virus control and ACV at ED<sub>50</sub> concentration.



**Figure 3.** Titer of HSV-2 at 0, 18, 24 and 30 h after treatment with methanol extract of lavender (LM) 19 µg/ml, sage (SM) 19 µg/ml and chamomile (CM) 313 µg/ml compared with untreated virus control and ACV at ED<sub>50</sub> concentration.



**Figure 4.** Titer of HSV-2 at 0, 18, 24 and 30 h after treatment with dichloromethane extract of lavender (LD) 9 µg/ml, sage (SD) 9 µg/ml and chamomile (CD) 156 µg/ml compared with untreated virus control and ACV at ED<sub>50</sub> concentration.

chamomile and the activity was more pronounced on HSV-2. The similar results were observed since *Barleria lupulina* and *Clinacanthus nutans* extracts could inactivate HSV-2 particles directly (Yoosook et al., 1999), whereas *Artemisia arborescens* essential oil, *Eugenia caryophyllus* extract and eugenol essential oil could inactivate both HSV-1 and HSV-2 particles (Saddi et al., 2007; Tragoolpua and Jatisatienr, 2007).

At 30 h after infection, the highest inhibitory activities of chamomile extracts were observed for both HSV-1 and HSV-2. The anti-HSV activity of chamomile extracts seemed to be retained only within a short period. This may be due to the instability of the active ingredient in the extract. Greater stability of the extracts was observed after treatment HSV-1 with dichloromethane extract of lavender, and treatment of HSV-2 with methanol and dichloromethane extract of sage. Thus, the extracts of chamomile, sage and lavender demonstrated efficient anti-HSV activities, although, the most effective extract was different at each stage of viral replication cycle. Beside this study, dichloromethane and methanol extracts of *Ocimum sanctum*, *Ocimum basilicum* and *Ocimum americanum* also showed anti-HSV activities at various steps of the viral multiplication cycle (Yucharoen et al., 2011).

Studies of *Dunbaria bella* Prain extract and fractions showed inhibition of HSV replication and viral release (Akanitapichat et al., 2006). *Lamiaceae* extracts were also demonstrated on HIV-1 replication by a virion-fusion assay (Geuenich et al., 2008). Many reports have indicated that isoborneol, a monoterpene from *Salvia fruticosa* essential oil, could inhibit glycosylation of HSV

polypeptides and showed virucidal activity against HSV-1 by almost 4 log<sub>10</sub> within 30 min of exposure (Armaka et al., 1999). Additionally, *Salvia* extracts showed antioxidant and antibacterial activities. *Salvia officinalis* L. essential oils showed inhibitory activity on *Escherichia coli*, *Salmonella typhi*, *Salmonella enteritidis* and *Shigella sonnei* (Bozin et al., 2007).

Apigenin 7-*o* -glucoside was found to be the major constituent of aqueous and methanolic extracts of chamomile against various human cancer cell lines.  $\alpha$ -bisabolol from chamomile was active against *Staphylococcus aureus*, *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Balazs and Tisserand, 1998; Carle et al., 1990; Shealy, 2002; Srivastava and Gupta, 2007).

Several studies of herbs have reported antioxidant activity of plants from the Lamiaceae family, which often contain large amounts of phenolic compounds (Kinoshita et al., 2007; Moreno et al., 2006). These activities include antibacterial (Moon et al., 2006; Moreno et al., 2006; Schelz et al., 2006) and anti-inflammatory properties (Della et al., 1990).

The data from this study will augment the results quoted from previous literature and thus may help develop therapeutic antiviral drugs from the aromatic herbs, chamomile, sage and lavender.

## Conclusion

Our findings demonstrate that chamomile extracts affected short term viral inhibition and also directly

inactivated viral particles. However, higher activity of the extracts was observed after treatment of HSV-1 with dichloromethane extract of lavender, and treatment of HSV-2 with methanol and dichloromethane extract of sage. Therefore, these aromatic plants; sage, lavender and chamomile, demonstrated therapeutic potential as anti-HSV agents.

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