Anti-Herpes Simplex Virus Type 2 of *Drymaria diandra* Blume Medicinal Plant

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ABSTRACT

Anti-herpes simplex virus type 2 of dichloromethane and methanol extracts of Thai medicinal plant, *Drymaria diandra* Blume was investigated on Green Monkey Kidney cells. Cytotoxicity tests performed by the MTT assay revealed that 50% cytotoxicity dose of dichloromethane and methanol extracts were 90±2 and 631±32 μg/ml respectively. In all experiments, non-toxic concentrations of the plant extracts were used to test the inhibitory effect on various steps of herpes simplex virus type 2 (HSV-2) multiplication cycle. HSV-2 particles were directly inactivated by the extracts since 1 hour of treatment. HSV-2 infection was inhibited by the methanol extract with the 50% effective dose of 166.6±23.1 μg/ml. Moreover, both extracts inhibited HSV-2 attachment and penetration. The extracts also showed potent inhibitory activities against viral deoxyribonucleic acid synthesis as determined by real time polymerase chain reaction. Western blot analysis showed that HSV-2 proteins, particularly those at approximately 45 kDa were inhibited by the extracts after detection by horseradish peroxidase-conjugated immunoglobulin G against HSV. Therefore, this study demonstrated the inhibitory efficacy of *Drymaria diandra* Blume extracts against herpes simplex virus infection as a potential anti-HSV agent.

Keywords: *Drymaria diandra* Blume, herpes simplex virus, anti-viral, plant.

1. INTRODUCTION

Herpes simplex virus or Human herpes-virus (HSV/HHV) is important viral pathogen in human and is relatively widespread in both developed and developing countries around the world [1-3]. The viral particles composed of a large double-stranded DNA which surrounded by an icosahedral capsid and further coated with a lipid envelope [4, 5]. HSV is a member of family *Herpesviridae*, subfamily *Alphaherpesvirinae* and can be divided into 2 types (HSV-1/HHV-1), and HSV-2/HHV-2).

The transmission of HSV-2 viral disease is dependent on intimate person-to-person contact with individual excreting HSV-2 from sexual partner leading to the development of primary genital herpes, which mostly affect adult people [6]. After primary infection, the viral nucleic acid is transported from the infected mucosal area by neurons to dorsal...
root ganglia where latency is established in the sacral ganglia [7]. Furthermore, latent virus may be reactivated causing recurrent infections by local stimuli such as injury to tissues or systematic stimuli such as physical or emotional stresses, menstruation and hormonal disturbance [3, 6, 8]. Several studies have indicated that the frequency of genital herpes as a high recurrence could be around 60% [9-11]. Anti-herpes virus drugs such as acyclovir (ACV) have been remarkably successful in HSV treatment. Acyclovir was first approved for clinical use in early 1980s. Its antiviral activity was discovered and biochemical studies of the mechanism of acyclovir against HSV and VZV have been undertaken since 1977 [12]. However, side effects and drug resistant strains, which affect about 5% of immunocompromised patients receiving long-term prophylactic treatment with ACV, may lead to ineffective therapy [7, 13]. Resistance to acyclovir and related nucleoside analogues can occur following mutation in either HSV thymidine kinase gene or DNA polymerase gene. Moreover, it should be considered that these drugs are expensive and patients with frequent attacks may not be able to afford the cost of long-term treatment [14]. For these reasons, new anti-viral agents exhibiting different mechanisms of action are urgently needed [1].

In Thailand, people have utilized traditional medicine as their major primary health care. Ethnobotany in northern Thailand was studied in minorities; Yunnan Chinese, Akha, Lisu, Lahu, and Mien who used over 500 species for a variety of purposes [15]. Hence, ethnopharmacology or ethnobotany in the case of medicinal plants can play a pivotal role in drug discovery by utilizing the impressive array of knowledge and wisdom of indigenous people about their ancient medicaments [16].

Drymaria diandra Blume is often found in the mountains, along watercourses up to 1,700 meters altitude. This medicinal plant has been used for draught and treating tinea by hill tribes in Thailand. Moreover, it is used for antipyretic and cathartic treatment, and relieving stomatitis [17,18]. Therefore, the variety of plants from hill tribe medicaments represents an opportunity to explore anti-herpes simplex virus type 2 and modes of inhibition of D. diandra Blume extracts were investigated in this study.

2. MATERIALS AND METHODS

2.1 Plant Material

Drymaria diandra Blume (Caryophyllaceae) plants were collected from Nahaew district, Loei Province, Thailand during December 2005-February, 2006. Voucher specimen (QSBG 10492) was deposited in the herbarium at Queen Sirikit Botanical Garden, Chiang Mai Province, Thailand.

2.2 Cell Line and Virus

Green monkey kidney cells (GMK) were grown in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated bovine calf serum (Hyclone) and 40 μg/ml gentamicin (Bio Basic Inc.), in a humidified 5% carbon dioxide (CO2) incubator at 37°C.

Herpes simplex virus types 2 strain G was used throughout the study. Quantitative of the virus was performed in 24-well tissue culture plates, using a plaque titration assay. Plaques were counted and the virus titers were expressed as plaque forming units (PFU)/ml

2.3 Plant Extracts

Dried plant leaves (632g) were milled and soaked in methanol or dichloromethane at room temperature for 3 days. The suspension was then filtered and the solvent was evaporated, using a rotary evaporator and dried in high vacuum. The dry material was dissolved in dimethyl sulfoxide (DMSO)
before determination of anti-HSV activity.

2.4 Cytotoxicity Test by 3-[4,5-dimethyl-thiazol-2-yl]-2,5 Diphenyl Tetrazolium Bromide (MTT) Assay

Cytotoxicity tests were performed following previously published procedures [19]. Cell toxicity was quantified using a MTT assay. Briefly, GMK cells were seeded on 96-well microplates and incubated with non-toxic concentrations of plant extracts for 72 hours. The media were removed before being incubated with MTT solution for 4 hours. Then, the blue formazan product was eluted from cells by adding DMSO. The absorbance at 570 nm was determined using a microplate reader. The concentration of the 50% cytotoxic dose (CD50) was calculated.

2.5 Inactivation Kinetics

HSV was treated with non-toxic concentrations of the extracts at room temperature. Aliquots of the virus-extract mixture were taken and diluted for residual virus assay at 1 hour intervals for up to 4 hours, using the plaque titration assay. The residual virus titers were determined from two independent experiments.

2.6 Plaque Reduction Assay

The GMK 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 incubated at room temperature for 1 hour. Then, 200 μl of crude extract of D. diandra Blume at non-toxic concentrations and acyclovir (ACV) at 50% effective dose (ED50) concentration were applied in duplicate test wells whereas the media were added into control wells. After that, 400 μl of growth media, containing 2% sodium carboxymethyl cellulose, were added to the cells. After 3 days incubation in the 5% CO2 incubator, the cells were stained with 0.1% crystal violet in 1% ethanol. The plaques were counted and the inhibitory activities of tested extracts were calculated comparing with the control. The ED50 was also determined.

2.7 Effect of Plant Extract on Viral Attachment

2.7.1 Supplement virus and extract at the same time

To determine inhibitory effect of the extract on viral attachment, GMK cell monolayers were pre-chilled at 4°C for 1 hour. The media were aspirated and the cell monolayers were infected with HSV in the absence or presence of either extracts or acyclovir. Then, the infected cells were further incubated at 4°C for 3 hours and the cells were washed with phosphate buffered saline (PBS) twice before overlaid with growth media, containing 2% sodium carboxymethyl cellulose [20]. After 2-3 days incubation, the cells were stained with 0.1% crystal violet in 1% ethanol. The percentage of inhibition for HSV attachment to GMK monolayer was calculated.

2.7.2 Effect of plant extract and heparin on the cell receptors

Effect of the plant extracts on the cell receptors was performed by the method modified from Lee and Lobigs, 2000 [21]. Plant extract was incubated with or without heparin (16 μg/ml) at 4°C for 1 hour. Then, 200 μl of HSV was added to the mixture and was further incubated at 4°C for 15 minutes. After that, the mixture was added to GMK cells and incubated at room temperature for 1 hour. Infected cells were washed twice with PBS, and then 500 μl of growth media containing 2% sodium carboxymethyl cellulose was added to the cells. After 2-3 days 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 extracts on HSV were calculated comparing to the inhibition of extracts with heparin and infected cell virus control.

2.8 Effect of Plant Extract on Viral Penetration

GMK cell monolayers were pre-chilled at 4°C for 1 hour [22]. HSV was inoculated on cell monolayers and infected cells were further incubated at 4°C for 3 hours. The extract or acyclovir was added and then incubated at 37°C for 10 minutes. Then, the infected cells were treated with PBS, pH 3 for 1 minute, and PBS, pH 11 was added immediately to neutralize acidic PBS. After that, PBS was then removed and the cell monolayer was overlaid with growth media, containing 2% sodium carboxymethyl cellulose and incubated for 3 days. The cells were stained with 0.1% crystal violet in 1% ethanol and the percentage of inhibition for HSV penetration was calculated.

2.9 Effect of Plant Extract on HSV Replication

GMK cells were grown as monolayers in 25 cm² flasks. The cells (6×10⁵ cells/ml) were infected with 1×10⁶ PFU/ml of HSV. Virus was allowed to adsorb to the cells for 60 minutes at room temperature. Unadsorbed virus was discarded and the cells were washed twice with PBS. The duplicated flasks of infected cells were maintained in the media containing extract of D. diandra Blume at non-toxic concentration while only media or media containing acyclovir at ED₅₀ concentration were added into control flasks. Infected cells were observed for cytopathic effects (CPE) and were collected at 6, 12, 24 and 30 hours after viral infection. The cells were frozen and thawed twice. The supernatants containing viruses were kept at -80°C and virus titers were determined using plaque titration assay.

2.10 Viral Deoxyribonucleic Acid (DNA) Extraction

GMK cells (4×10⁶ cells/ml) were infected with HSV with a multiplicity of infection (MOI) of 1 in the presence or absence of the extracts. The infected cells were harvested when 80-90% of infected cell showed cytopathic effect. The cells were lysed with lysis solution (0.25% Triton X-100, 0.5M EDTA and 1M Tris-HCl, pH 8.0). Then, the cell lysate was treated with 5M NaCl, proteinase K and RNase A. The viral DNA was extracted with phenol-chloroform-isoamyl alcohol and precipitated with cold absolute ethanol. The concentration of viral DNA was determined after measuring the optical density at 260 nm. Viral DNA was then quantitatively detected by real-time PCR.

2.11 Quantitative Real-Time Polymerase Chain Reaction (PCR)

Amplification and detection of viral DNA by real-time PCR was carried out using the iCycler iQ (Bio-Rad, Hercules, CA). Amplified 1,350 bp region of viral DNA polymerase gene was produced. A total volume of 25 μl was obtained by adding 1 μl of viral DNA to PCR reaction. Forward primer (5′-ATGCAGAAGCAGATCCGCTGCG-3′) and reverse primer (5′-GGCTCTATGCAATTCGACG-3′) were used at 0.5 μM. PCR products were detected using the sequence unspecific SYBR Green I dye (Maxima™ SYBR Green qPCR Master Mix (2x); Fermentas) with the melting curve analysis.

PCR conditions were as follows. Activation of SYBR green I was performed at 95.0°C for 10 minutes followed by 40 cycles of denaturation at 95.0°C for 45 seconds, annealing at 60.0°C for 45 seconds and extension at 72.0°C for 3 minutes, then, melting curve program was used at 55-95°C with increase set point temperature after two
cycles by 0.5°C and cooling temperature at 20°C. After cycle completed, a threshold for detection of fluorescence above background is determined by iCycler iQ software. The cycle at which the fluorescence from a sample crosses the threshold is called the cycle threshold, C_t. The quantity of DNA doubles every cycle during the exponential phase; therefore, relative amounts of DNA can be calculated.

2.12 Western Blot Analysis

GMK cells were grown as monolayer in 25 cm² flasks (4×10⁵ cells/ml). The cells were infected with HSV with MOI = 1. Virus was allowed to adsorb to the cells for 60 minutes at room temperature. Unadsorbed virus was discarded and the cells were washed twice with PBS. Infected cells were maintained in the media containing extracts of D. diandra Blume at non-toxic concentrations while only media were added into the control flasks. The infected cells were observed for CPE and were collected at 24 hours after infection. Total proteins were extracted from the infected cells using NucleoSpin® RNA/Protein (MACHEREY-NAGEL) and ten microliter of total protein extracts were detected by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then, the protein was transferred to nitrocellulose filter and the filter was blocked by soaking overnight in 3% bovine serum albumin in saline [23]. The blotting filters were incubated overnight with horseradish peroxidase-conjugated immunoglobulin G against HSV (AbD serotec) and washed in saline 5 times for 30 minutes. HSV protein bands were detected by incubating the blotting filter in 0.06% 4-chloro-1-naphthol (sigma)/ 0.01% H₂O₂ in PBS and then washed in deionized water to stop reaction.

2.13 Identification of Compounds in D. diandra Blume Extracts

The extracts were sent for compound identification at Central Laboratory (Thailand) Co., Ltd.; Thailand using GC-MS scan compared with Wiley version 7.0 library database (>70% matched).

2.14 Data Analysis

The CD₅₀ and ED₅₀ are calculated from two independent experiments, and selective index (SI) is defined as CD₅₀/ED₅₀. Program Gene Tool (Image Analysis System) was used for analyzing protein bands quantitatively.

3. RESULTS AND DISCUSSION

HSV infection in some people can be kept under control with synthetic anti-HSV drugs but these expensive drugs are still far beyond the means of most developing countries. Therefore, requirement for the safe, effective and inexpensive antiviral drugs are needed [24]. Many reports indicated that compound isolated from medicinal plants can suppress the multiplication of HSV [25-27]. Highland medicinal plant in northern Thailand, Cissus repanda Vahl showed inhibitory activity against HSV type 1 and type 2 [28]. Thus, research in medicinal plants and ethnopharmacology can serve as an alternative approach for the discovery of novel antiviral agents. HSV-2 inhibition by D. diandra Blume extract was investigated in this study. The extracts affected HSV particle directly and also inhibited HSV infection during attachment and penetration. Moreover, the extracts affected viral DNA replication and protein synthesis.

D. diandra Blume was extracted using dichloromethane (DD) and methanol (DM). The crude extracts were examined for cell cytotoxicity by MTT assay (Table 1). CD₅₀ of DD and DM extracts were 90±2 and 631±32 μg/ml, respectively, thus DD extract showed higher toxicity on GMK cells as observed from concentration of the extract that caused
cells death by 50%. Therefore, non-toxic concentrations of DD and DM extracts at 35 and 272 μg/ml were used throughout the experiments.

Both extracts; DD (35 μg/ml) and DM (272 μg/ml) showed the ability to inactivate HSV-2 strain G directly within 1 hour of incubation (Table 2). Besides this plants, Barleria lupulina and Clinacanthus nutans extracts could inactivate HSV-2 directly [29]. The virucidal activities of B. lupulina appeared to be much higher than C. nutans extracts. Moreover, crude extract of E. caryophyllus were able to inactivate HSV-2 strain G within 3 hours [30].

Studies on antiviral activities of medicinal plants have been performed using an in vitro experiment. Among various assay methods, plaque reduction assay has always been used for detecting antiviral activities of both synthetic and natural products [31]. The antiviral agents were incorporated into the overlay medium or added after virus adsorption.

In this study, plaque reduction assay showed that DM extract inhibited HSV-2 with ED₅₀ of 166.6±23.1 μg/ml and the SI value (CD₅₀/ED₅₀) was 3.8±0.5 whereas the percentage of HSV inhibition of DD extract was less than 50% (Table 1). In addition, plaque sizes after HSV infection were reduced after treatment with DM extract compared with HSV infected cells. It revealed that these extracts may 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 that virucides or early-acting antivirals may behave differently, depending on treatment schedule and the ability of the virus to spread via syncytia.

In the yield reduction assay, the effect of extracts on the production of viral progenies was determined. Thirty hours after HSV-2

<table>
<thead>
<tr>
<th>Virus</th>
<th>Extracts</th>
<th>Concentration (μg/ml)</th>
<th>CD₅₀ ± SD (μg/ml)</th>
<th>ED₅₀ ± SD (μg/ml)</th>
<th>SI ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2</td>
<td>DD</td>
<td>35</td>
<td>90 ± 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>272</td>
<td>631 ± 32</td>
<td>166.6 ± 23.1</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>acyclovir</td>
<td>-</td>
<td>-</td>
<td>12.8 ± 0.8</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. of two independent experiments.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Extracts</th>
<th>Concentration (μg/ml)</th>
<th>Log amount of viruses (PFU/ml) at one hour interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2</td>
<td>DD</td>
<td>35</td>
<td>0  1    2  3  4</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>272</td>
<td>0  1    2  3  4</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>5.7</td>
<td>4.3 &lt;1 &lt;1 &lt;1 &lt;1</td>
</tr>
</tbody>
</table>

Table 1. Cytotoxicity dose (CD₅₀), anti-HSV activity and selective index (SI) of the extracts on GMK cells.

Table 2. Direct inactivation of HSV-2 by dichloromethane and methanol extracts of D. diandra Blume.
replication, the reduction of viral amounts was 51.9% and 100% after treatment with DD and DM extracts respectively (Figure 1). Percentage of viral inhibition observed by yield assay within 30 hours post infection was higher than that observed by plaque reduction assay, which was evaluated after 72 hours post infection. It was also found that the extracts showed less activity when treated for long time, implying that the possible instability of ingredients in the extracts may influence antiviral activity. The similar result was found in the study of quercetin compound. The compound was found to be effective when the time of treatment of virus was around 24 hours or approximately one multiplication cycle and the compound was considered ineffective when the treatment was longer for 3 days [32]. Furthermore, Prichard et al. (1990) [33] suggested that the yield reduction showed a better reflection of the strategies in infected hosts than the plaque inhibition assay. Despite this fact, the yield reduction assay has not been widely used for screening antiviral property due to labor intensive nature of the method.

Mode of inhibition of HSV infection was performed on viral attachment, penetration, viral DNA replication and viral protein expression in this study. Inhibitory effect of the extract on viral attachment was determined when supplement the virus and extract at the same time and the percentages of HSV-2 inhibition were 95.8±1.5 and 97.4±3.7 after treatment DD and DM extract respectively (Figure 2). Effect of plant extract on HSV penetration was also investigated in this study. DD and DM extracts showed percentage of inhibition by 59.6±5.7 and 72.7±4.3, respectively (Figure 2). It displayed that both extracts could block the binding of viral glycoproteins to cellular receptors, and viral penetration. However, the effect of DM extract on HSV was more than DD extract.
when applied during viral penetration and viral replication. The effect of DM and DD extracts was not different when observed on direct inactivation of HSV particles and during viral attachment. This may due to the different potential of compound in each DM or DD extract that act better against viral particles before penetration to the cells and the activity was still remained only in DM extract during viral penetration and replication.

**Figure 2.** Effect of DM and DD extracts on HSV-2 attachment and penetration to GMK cells. The percentage of inhibition of the extracts was evaluated by plaque assay. Each bar represents the mean ± S.D. for two independent experiments.

Therefore, the effect of the extracts on heparan sulphate receptor molecule was determined. Heparin was selected in this study as a useful model for heparan sulfate receptor binding molecule for HSV attachment. Heparin is a member of the glycosaminoglycan family of carbohydrates, which includes the closely-related molecule heparan sulfate [34]. Normally, heparan sulfate exists on the surface of most mammalian cells. The main repeat unit of heparin structurally resembles the protein binding sequences in heparan sulfate, but contains a higher percentage of sulfated residues [35, 36]. Thus, DD and DM extracts were mixed with or without heparin and viruses, and then added on the cells. It was found that after treatment HSV with the mixture of extract and heparin showed higher percentage of viral inhibition than the treatment with extract alone and heparin control (Table 3).

It predicted that DD and DM extracts might not affect on blocking heparan sulfate on cell membrane that interact with HSV glycoprotein C or B [37]. The extracts might block other cellular receptors that involved in viral attachment and resulted in blocking HSV receptor binding, and further inhibited HSV.
Another possibility was that activity of extracts on HSV might enforce by mixing with heparin since the percentage of inhibition during viral attachment by DD and DM extracts was increased during treatment HSV attachment with the mixture of the extracts and heparin. However, further experiment should be performed to clarify this explanation.

Inhibition of HSV attachment and penetration were also shown from other studies. Pterocarnin A extracted from the bark of *Pterocarya atenoptera* showed inhibitory activity against HSV-2 during attachment and penetration into cells [20], and casuarinin extracted from the bark of *Terminalia arjuna* Linn. interfered with the attachment of HSV-2 into cells [22].

In addition, Quantitative real-time PCR was performed to detect HSV-2 DNA replication on GMK cells in the presence or absence of the extract using designed primer specific to HSV DNA polymerase gene. PCR products were detected at 1350 base pair using SYBR Green I dye. HSV DNA after treatment with the extracts was 1.4 to 1.9 fold less than HSVs DNA control as observed by crossing point values or cycle threshold, $C_t$ (Figure 3). The values were calculated directly as the coordinates of points in which the threshold line actually crossed [38]. High $C_t$ value reflected many cycles of PCR to

**Table 3.** The percentage of inhibition of the effect of the extract and heparin on the HSV-2 attachment.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Extracts</th>
<th>Mean % inhibition ± SD$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2</td>
<td>DD mixed with heparin</td>
<td>94.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>DM mixed with heparin</td>
<td>97.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>87.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>90.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>68.8 ± 2.4</td>
</tr>
</tbody>
</table>

$^a$Values are means of two independent experiments

![Figure 3. Effect of DD and DM extracts on HSV-2 DNA accumulation in infected cell.](image)
generate DNA product. Hence, it indicated that *D. diandra* Blume extracts may interfere with HSV DNA synthesis by affecting HSV DNA replication directly or may inactivate essential enzymes for viral DNA synthesis e.g. origin-binding protein (*UL9* gene product), single-stranded binding protein (*UL29* gene product), DNA polymerase (*UL30/UL42* gene product) or helicase-primase (*UL5/UL52/UL8* gene product) [39-41].

Viral protein expression was also inhibited by the extract. Approximately 45 kDa HSV-2 proteins were clearly diminished after treatment with DM extracts (Figure 4). The 45 kDa viral protein was likely to be VP22a and the extracts could reduce the amount of this protein. VP22a is required as scaffold for assembly of viral capsid. In the absence of the scaffolding function, the proteins that form the outer shell assemble into aberrant structures [42-45]. In addition, other viral proteins might be blocked by the extract.

Compounds in DD and DM crude were identified at Central Laboratory (Thailand) Co., Ltd., Thailand using GC-MS scan compared with Wiley version 7.0 library database (>70% matched). It was found that compounds in DD extract were pentadecanone, hydroxymethylhydrofuranone, palmitic acid (ester), palmitic acid and stearic acid, 9, 12, 15-octadecatrien-1-ol, hexadecic acid, palmitic acid, 2-methyl-octadecyne and linoleic acid 2-hexadecen-1-ol, linolic acid, 3, 7, 11, 15-

![Figure 4](image)

**Figure 4.** Effect of the DD and DM extracts on HSV-2 viral protein synthesis compared with the control. Western Blots of uninfected cells (lane 1), the presence of DD extract (lane 2), DM extract (lane 3) and absence of the extract (lane 4) after detection by horseradish peroxidase-conjugated IgG against HSV compared with protein marker. Arrow heads indicate the reduction of viral proteins after treatment with the extracts.
tetramethyl-2-hexadecen-1-ol, 2-pentadecanone, 2-heptanone, 1,4-eicosadiene and 2-methyl-octadecyne. Compounds in DM extract were identified as 4-cyclopentene-1,3-dione, protoanemonine, hexadecanoic acid, palmitic acid, pentadecanecarboxylic acid, 1-pentadecanecarboxylic acid, cetylic acid, 7-pentadecyne, 9, 12, 15-octadecatrien-1-ol, 7, 10, 13-hexadecatrienoic acid, dioctyl ester, dioctyl adipate and hexanedioic acid. These components might have synergistic and additive effects against HSV. The previous report showed anti-human immunodeficiency virus alkaloid, drymaritin was isolated from D. diandra [46]. However, the active compounds from D. diandra Blume, which conferred anti-HSV activity, should be further studied.

4. CONCLUSIONS

Dichloromethane and methanol extracts of D. diandra Blume demonstrated anti-HSV-2 activity and HSV particles was directly inactivated within 1 hour by the extracts. The extracts showed inhibitory activity against HSV infection during attachment and penetration. Inhibition during viral attachment by the extracts was increased during treatment HSV attachment with the mixture of the extracts and heparin. Moreover, viral DNA replication as determined by real time polymerase chain reaction and HSV proteins particularly those at approximately 45 kDa were also inhibited by the extracts. However, the active compounds, which conferred anti-HSV activity by the D. diandra Blume plant extracts, should be further studied and the efficacy as a potential anti-viral agent should be tested for treatment of HSV infection.

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