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Antiviral activity of *Plantago major* extracts and related compounds in vitro

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Abstract

Plantago major L., a popular traditional Chinese medicine, has long been used for treating various diseases varying from cold to viral hepatitis. The aim of present study was to examine the antiviral activity of aqueous extract and pure compounds of *P. major*. Studies were conducted on a series of viruses, namely herpesviruses (HSV-1, HSV-2) and adenoviruses (ADV-3, ADV-8, ADV-11). The antiviral activity of EC_{50} was defined as the concentration achieved 50% cyto-protection against virus infection and the selectivity index (SI) was determined by the ratio of CC_{50} (concentration of 50% cellular cytotoxicity) to EC_{50}. Results showed that aqueous extract of *P. major* possessed only a slight anti-herpes virus activity. In contrast, certain pure compounds belonging to the five different classes of chemicals found in extracts of this plant exhibited potent antiviral activity. Among them, caffeic acid exhibited the strongest activity against HSV-1 ($EC_{50} = 15.3$ μg/ml, SI = 671), HSV-2 ($EC_{50} = 87.3$ μg/ml, SI = 118) and ADV-3 ($EC_{50} = 14.2$ μg/ml, SI = 727), whereas chlorogenic acid possessed the strongest anti-ADV-11 ($EC_{50} = 13.3$ μg/ml, SI = 301) activity. The present study concludes that pure compounds of *P. major*, which possess antiviral activities are mainly derived from the phenolic compounds, especially caffeic acid. Its mode of action against HSV-2 and ADV-3 was found to be at multiplication stages (postinfection of HSV-1: 0–12 h; ADV-3: 0–2 h), and with SI values greater than 400, suggesting the potential use of this compound for treatment of the infection by these two viruses.

Keywords: *Plantago major*; Caffeic acid; Chlorogenic acid; Herpes simplex virus; Adenovirus; Phenolic compound

1. Introduction

Virus infection is a common worldwide problem. Herpes simplex virus infection is found in over 60 million people in the US, most of whom are of childbearing age (Whitley, 1994; Corey, 1994).

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Several drugs have been approved by the Food and Drug Administration for treatment of viral infections, of which most of them were synthetic nucleoside analogues (Wood, 1999). Resistance of virus to chemotherapy has been reported to develop in vitro and in vivo. In general, drug resistance is limited to patients who are immunocompromised (Reusser, 2000; Snoeck, 2000; Field, 2001). It is, therefore, necessary to find new alternative antiviral compounds. Pure compounds of plant origin have been shown to exhibit antiviral activity against herpes simplex virus. Phytochemicals possessing such activity are alkaloids (Martin, 1987), flavonoids (Lin et al., 1999), saponins (Sindambiwe et al., 1998), quinones (Andersen et al., 1991), terpenes (Bourne et al., 1999), lignans (Charlton, 1998), tannins (Ferrea et al., 1993), polysaccharides (Bourne et al., 1999), steroidal glycoside (Ikeda et al., 2000), thiosulfinates (Tasi et al., 1985), proanthocyanidin (Erdelmeier et al., 1996) and proteins (Aoki et al., 1995).

Adenoviral infections can occur throughout the year in all age groups. It can be either an outbreak or a sporadic case in many countries. Outbreaks of eye illness usually occur in the summer, but respiratory infections are the highest during the period between the autumn and the winter. Illnesses associated with human adenoviruses (ADV) involve ocular, respiratory, gastrointestinal, urinary, or genital organs. Adenoviral pneumonia has been reported to have considerable mortality rate especially in children of age below 2 years old (Dudding et al., 1972; Straube et al., 1983; Avila et al., 1989). Severe ADV infections occur mainly in immunocompromised patients, i.e. patients with leukemia (Zahradnik et al., 1980), or AIDS (De Jong et al., 1983), and organ transplantation (Stalder et al., 1977). 5-Iodo-2-deoxyuridine has been used in the chemotherapy of ADV infection (Dudgeon et al., 1969). Several investigators reported that some modified nucleoside analogues, i.e. 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl] purine (S-2242), (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine [(S)-HPMPA], (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine [(S)-HPMPC], 3′-Fluoro-2′-deoxythymidine (FTdR), 3′-fluoro-2′-deoxyuridine (FudR), 2′,3′-dideoxycytidine (ddC), 2′-nor-cyclic GMP and 3′-fluoro-2′-deoxyguanosine (FGdR) were effective in inhibiting ADV replication (Gordon et al., 1991; Kodama et al., 1996; Mentel et al., 1997). A recent report has shown that the cysteine protease inhibitors were effective against the adenovirus infection in tissue culture (Sircar et al., 1996). However, there is no approved chemotherapy effective in preventing or interrupting this virus infection.

Plantago major L. is a perennial plant that belongs to the Plantagiaceae family. This is a commonly used medicinal herb in Taiwan, which has been used in the treatment of a number of diseases related to the skin, respiratory organs, digestive organs, reproduction, circulation, cancer prevention and against infections (McCutcheon et al., 1995). It has also been used as a remedy for colds and viral hepatitis (Gupta et al., 1979; Yu and Xu, 1989; Lin and Kan, 1990). The ethanol or methanol extract of the entire plant was reported to have no antiviral activity (McCutcheon et al., 1995). This plant has shown to contain five classes of biologically active compounds, namely benzoic compound (vanillic acid), flavonoids (baicalein, baicalin, luteolin), iridoid glycoside (aucubin), phenolic compounds (caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid) and triterpenes (oleanolic acid, ursolic acid) (Duke 1992; Samuelsen, 2000). Its ferulic acid and caffeic acid have been demonstrated to possess activity against herpes simplex virus 2 (HSV-2) in vitro (Bourne et al., 1999). This traditional Chinese herbal remedy has been used for treating colds, conjunctivitis and hepatitis for hundreds of years in Taiwan. In our preliminary test, aqueous extract of *P. major* was found to possess activity against HSV-2. This result, therefore, led us to select the main five classes of chemicals from *P. major* for evaluating antiviral activity. In this paper, our objective was to evaluate the antiviral activity of aqueous extract and pure compounds of *P. major* against a series of viruses, namely herpes simplex virus (types: HSV-1, HSV-2) and adenovirus (types: ADV-3, ADV-8, ADV-11).

2. Materials and methods

2.1. Extract and pure compounds

Aucubin, baicalein, baicalin, chlorogenic acid and luteolin were purchased from Wako Pure Chemical Industries, Ltd (Japan). Acyclovir, 2′3′-dideoxycytidine (ddC), dimethylsulfoxide (DMSO), caffeic acid, ferulic acid, oleanolic acid, *p*-coumaric acid, ursolic acid and vanillic acid were obtained from Sigma Chemical Co (USA). XTT kits were purchased from Roche Diagnostics GmbH (Germany).

The whole plant of *P. major* was collected from the southern part of Taiwan. Its authenticity was identified and confirmed using morphological and anatomical techniques by Professor Chun-Ching Lin, Graduate Institute of Natural Products, Kaohsiung Medical University. A voucher specimen of the plant was deposited at the Herbarium of the Graduate Institute of Natural Products of Kaohsiung Medical University. Hot water extract of *P. major* was prepared from the entire plant according to the standard methods with minor modification as previously reported (Chang and Yeung, 1988). In brief, dried crude drugs (100 g) were boiled in 1000 ml of distilled water for 1 h, the decoction obtained was then filtered by gauze. The same procedure was repeated three times. The aqueous extract of three successive extraction was collected, combined and concentrated in vacuo, and then lyophilized. The crude dried extract was dissolved in distilled water and the pure compounds were suspended in DMSO.

2.2. Virus and cells

The human skin basal cell carcinoma cell line (BCC-1/KMC), which was established in our laboratory (Chiang et al., 1994), was used as target cells for virus infection in the XTT assay. It was derived from undifferentiated carcinoma cells and growth as adherent cells in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin G, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B. In the antiviral assay, the medium was supplemented with 2% FCS and the above mentioned antibiotics. The reagents for cell culture were obtained from GIBCO (Grand Island, NY).

The strain of HSV type 1 (HSV-1 strain KOS) used in this study was obtained from the American Type Culture Collection (ATCC), Rockville, MD. HSV-2 strain 196 was kindly provided by Dr W.T. Liu, School of Medical Technology, National Yang-Ming University. The clinical isolates of adenovirus (ADV), ADV-3, ADV-8 and ADV-11, were provided by K.H. Lin, Kaohsiung Medical University Hospital. HSV and ADV were propagated in BCC-1/KMC cells. Virus titers were determined by cytopathic effect in BCC-1/KMC cell and were expressed as 50% tissue culture infective dose ($TCID_{50}$) per ml. All viruses were stored at -70 °C until use.

2.3. Cellular toxicity

The BCC-1/KMC cells were seeded onto 96-well plate with a concentration of 1.0×10^5 cells per ml and a volume of 90 μl per well. Different concentrations of crude aqueous extract or pure compounds were applied to culture wells in triplicate. DMSO was used as control. After incubation at 37 °C with 5% CO_2 for 3 days, the mixture of 0.1 ml Phenazine methosulfate (PMS: electron-coupling reagent) and 5 mg/5 ml XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino) carbonyl-2H-tetrazolium hydroxide]) were added to each well with a volume of 50 μl. The trays were further incubated for 2 h to allow XTT formazan production. The optical densities were determined with the ELISA reader (Multiskan EX, Labsystems) at a test wavelength of 450 nm and a reference wavelength of 690 nm.

Data were calculated as percentage of inhibition by the following formula:

$$\text{Inhibition } \% = \left[100 - \left(\frac{\text{ODt}}{\text{ODs}}\right) \times 100\right]\%$$

ODt and ODs indicates the optical density of the test substances and the solvent control, respectively. The concentration of 50% cellular cytotoxicity (CC_{50}) of test substances was calculated according to Weislow et al. (1989).

2.4. Antiviral assay using XTT method

The antiviral activity of *P. major* and related compounds against HSV-1, HSV-2, ADV-3, ADV-8, ADV-11 were evaluated by the XTT method. BCC-1/KMC cells, treated by trypsin, were seeded onto 96-well plate with a concentration of 1.0×10^5 cells per ml and a volume of 70 μl per well. After incubation at 37 °C with 5% CO_2 for 6 h, 20 μl test virus was added and incubated for another 2 h. Different concentrations of test substances were then added to culture wells in triplicate. The maximum concentration of DMSO (0.1%) was used as negative control. Acyclovir and ddC were used as a positive control for HSV and ADV assays, respectively. After incubation at 37 °C with 5% CO_2 for 3 days, XTT test was done as previously described.

Viral inhibition rate was calculated as (ODtv − ODcv)/(ODcd − ODcv) × 100%.

ODtv indicates the absorbance of the test compounds with virus infected cells. ODcv and ODcd indicates the absorbance of the virus control and the absorbance of the cell control, respectively. The antiviral concentration of 50% effectiveness (EC_{50}) was defined as the concentration achieved 50% cyto-protection against virus infection (Kodama et al., 1996). The number of virus used in each experiment was based on infected target cells of 20–200 $TCID_{50}$ (MOI of 0.002–0.025) of HSV or ADV to produce 50% XTT formazan products as in uninfected control cells (Weislow et al., 1989).

2.5. Dose–response

HSV-1 (25 $TCID_{50}$ per well) was absorbed onto confluent monolayers of BCC-1/KMC cells for 2 h. Different concentrations of caffeic acid were added to culture cells in triplicate at 0, 1 or 2 h after virus infection. After 3 days, XTT test and antiviral activity were done as previously described.

2.6. Time course

Various concentrations of caffeic acid were added to culture cells in triplicate at different time of preinfection or postinfection. HSV-1 (25 $TCID_{50}$ per well) or ADV-3 (120 $TCID_{50}$ per well) was inoculated onto confluent monolayers of BCC-1/KMC cells for 2 h. After 3 days, XTT test and antiviral activity were done as previously described.

2.7. Statistical analysis

The selectivity index (SI) was determined by the ratio of CC_{50} to EC_{50}. The statistically different effects of test compounds on the inhibition of HSV or ADV replication were compared with the control group using the Student's *t*-test.

3. Results

3.1. Assessment of antiviral activity

Table 1 shows the antiviral activity of crude aqueous extract and pure compounds of *P. major*. With the exception of HSV-2, aqueous extract of *P. major* ($\leqq 1000$ μg/ml) failed to show any activity against HSV-1, ADV-3, ADV-8 and ADV-11. Among the pure compounds tested, chlorogenic acid and caffeic acid were found to possess the strongest antiviral activity; chlorogenic acid was active against HSV-1, HSV-2, ADV-3, ADV-8 and ADV-11, whereas caffeic acid was active against HSV-1, HSV-2 and ADV-3. Besides aucubin, luteolin, oleanolic acid and ursolic acid, which showed no antiviral activity in both herpes and adeno viruses, baicalein, baicalin, ferulic acid, *p*-coumaric acid and vanillic acid were active against at least one of the viruses tested. Interestingly, the effect of chlorogenic acid ($EC_{50} = 13.3 \pm 3.2$ μg/ml; SI = 301) possessed similar antiviral activity but a lower cytotoxicity than the standard drug (ddC: $EC_{50} = 14.0 \pm 0.1$ μg/ml; SI = 16.4) in inhibiting the ADV-11. In the adenovirus study, it was found that chlorogenic acid was active against ADV-3, ADV-8 and ADV-11; ferulic acid against ADV-8 and ADV-11; caffeic acid against ADV-3 and *p*-coumaric acid against ADV-11. Anti-HSV-1 activity was also noted in baicalein (Table 1).

Table 1
The assessment of antiviral activity of aqueous extract and pure compounds of *P. major*

Compound	Herpesviruses					Adenoviruses					
	HSV-1			HSV-2		ADV-3		ADV-8		ADV-11	
	CC_{50}[a]	EC_{50}[b]	SI[c]	EC_{50}	SI	EC_{50}	SI	EC_{50}	SI	EC_{50}	SI
Standard[d]		1.9	94	1.5	118	5.3	43.0	10	23	14	16.4
Aqueous extract	1809	>1000[e]		843	2.2	>1000		>1000		>1000	
Aucubin	52 720	>200		>200		>200		>200		>200	
Baicalein	19.5	4.7	4.2	>20		>20		>20		>20	
Baicalin	41.1	>50		61.5	0.7	>50		>50		>50	
Caffeic acid	10 293	15.3	671	87.3	118	14.2	727	>200		>200	
Chlorogenic acid	3995	47.6	83.9	86.5	46.2	76.0	52.6	108	36.9	13.3	301
Ferulic acid	92.6	>100		>100		>100		52.5	1.8	23.3	4.0
Luteolin	23.1	>25		>25		>25		>25		>25	
Oleanolic acid	37.8	>40		>40		>40		>40		>40	
p-coumaric acid	489	>200		32.8	14.9	>200		>200		43.8	11.2
Ursolic acid	14.4	>20		>20		>20		>20		>20	
Vanillic acid	1338	88.1	15.2	>200		>200		>200		>200	

[a] The 50% cytotoxic concentration of target cell (BCC-1/KMC) in μg/ml.
[b] Concentration of compound in μg/ml producing 50% inhibition of virus-induced cytopathic effect of three separate experiments.
[c] Selectivity index (SI) = CC_{50}/EC_{50}.
[d] CC_{50}: Acyclovir (HSV: 179), ddC (ADV: 229).
[e] Maximum concentration of compound to test did not find EC_{50}.

Compared with the standard (acyclovir), both caffeic acid ($EC_{50} = 15.3$; SI = 671) and chlorogenic acid ($EC_{50} = 47.6$; SI = 83.9) showed interesting activity against the herpes virus. Based on the SI values, the activity of the various pure compounds against HSV-1 is in the order of caffeic acid > chlorogenic acid > vanillic acid and for the HSV-2, the order is caffeic acid > chlorogenic acid > *p*-coumaric acid.

As caffeic acid was found to possess the strongest anti-viral activity (low EC_{50} and high SI values), study was therefore conducted to evaluate the dose–response effect of this compound towards the HSV-1-infected BCC-1/KMC cells. The result showed that the effect of caffeic acid exhibited an obvious dose-dependent effect on the virus (Fig. 1). This response continued to be noted even 1–12 h after infection.

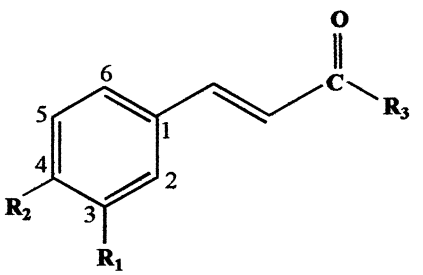
3.2. Time course

In order to investigate the mechanism on how caffeic acid inhibits the infection of herpes and adeno viruses, a study was conducted to investigate the time course effect at 2 h before and 24 h after the viruses infection and in contact with various concentrations of caffeic acid. The results showed that, caffeic acid at concentrations ≥ 20 μg/ml exhibited the greatest inhibition against HSV-1 infection from 0 to 12 h (Fig. 1). However, the inhibitory effect of caffeic acid on ADV-3 mainly occurred between 0 and 2 h, which was during the early period of virus replication (Fig. 2).

3.3. Comparison of phenolic compounds for antiviral activity against herpesviruses and adenoviruses

Table 2 shows the antiviral activity of phenolic compounds against herpesviruses and adenoviruses. In all of the phenolic compounds tested, the cinnamic acid is their common structure. However, compounds that contained hydroxyl group at the R_1 and R_2 positions (caffeic acid, chlorogenic acid) were found to possess more potent antiviral activity than compounds containing one hydroxyl group at the R_1 position (ferulic acid, *p*-coumaric acid).

Table 2
Structures of phenolic compounds and their antiviral activity



Compound	Antiviral activity								
	R_1	R_2	R_3	HSV-1	HSV-2	ADV-3	ADV-8	ADV-11	Ratio
Caffeic acid	OH	OH	OH	+[b]	+	+	−[c]	−	3/5
Chlorogenic acid	OH	OH	[a]	+	+	+	+	+	5/5
Ferulic acid	OH	OCH_3	OH	−	−	−	+	+	2/5
p-Coumaric acid	OH	H	OH	−	+	−	−	+	2/5

[a] 1,3,4,5-Tetrahydroxycyclohexane carboxylic acid.
[b] Concentration of compound <110 μg/ml producing 50% inhibition of virus-induced cytopathic effect.
[c] Concentration of compound >110 μg/ml not producing 50% inhibition of virus-induced cytopathic effect.

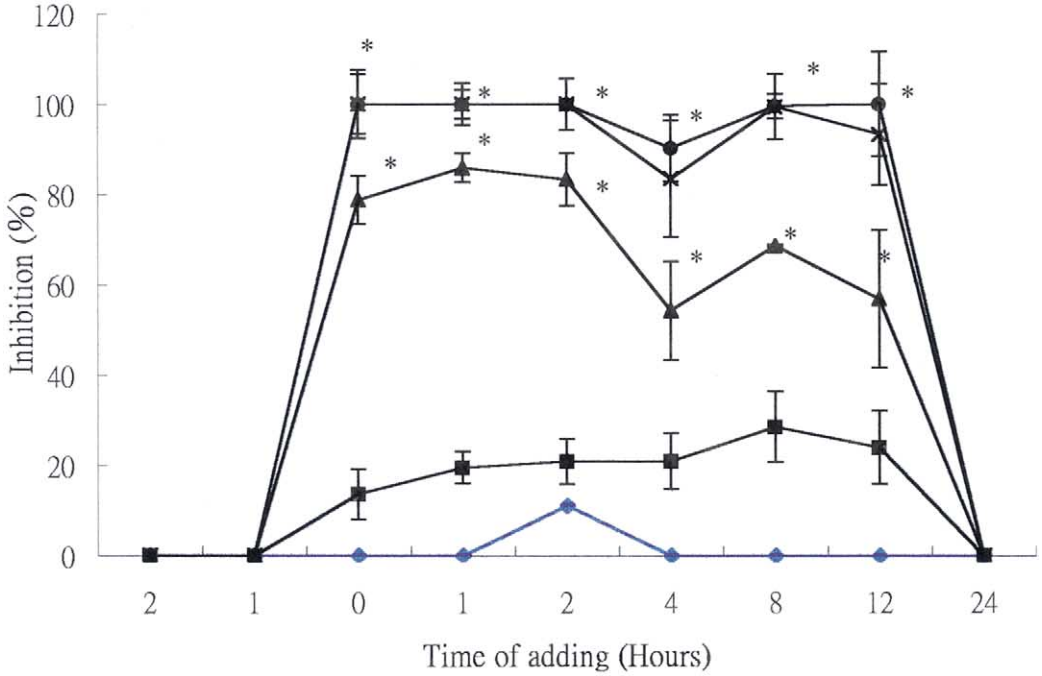


Fig. 1

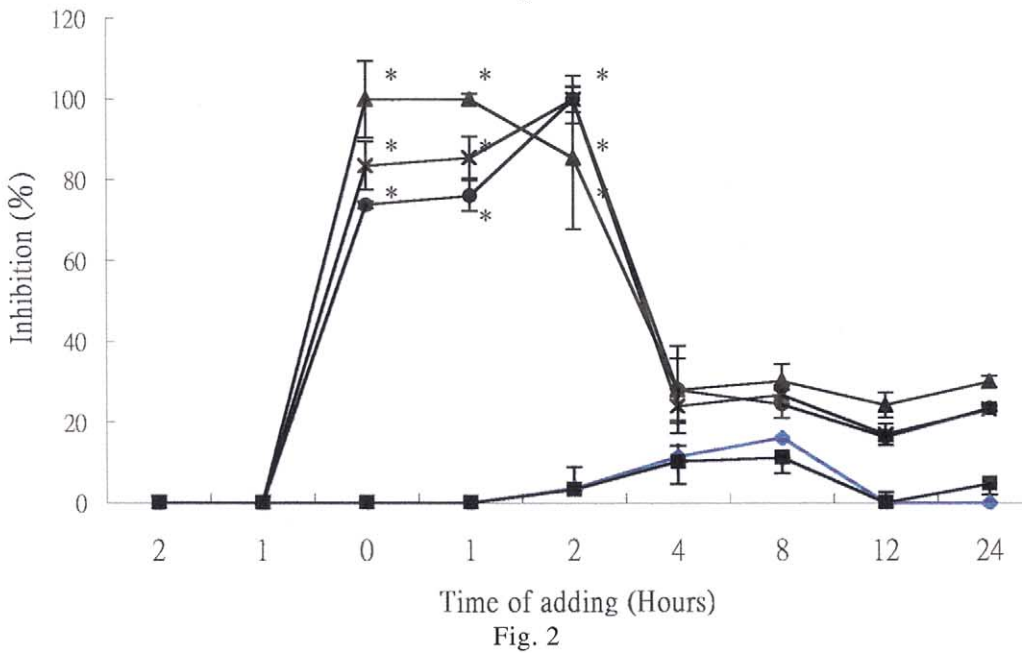


Fig. 2

Fig. 1. Inhibitory effect of adding caffeic acid at various time of preinfection or postinfection of herpesvirus (HSV-1) to BCC-1/KMC cells. Different concentrations of caffeic acid [1 μg/ml (◆), 5 μg/ml (■), 20 μg/ml (▲), 40 μg/ml (X), 50 μg/ml (●)] were added at various time of preinfection (-2, -1 h), coinfection (0 h) or postinfection (1–24 h) of herpesvirus (HSV-1) to BCC-1/KMC cells at 37 °C. After 3 days, optical density was evaluated by XTT method and expressed as the inhibition rate. The x-axis indicates the time course of adding caffeic acid. Each point represents the mean ± S.E. of triplicate samples of three independent experiments. The asterisk (*) indicates significant difference between test and DMSO control ($P < 0.01$).

Fig. 2. Inhibitory effect of adding caffeic acid at various time of preinfection or postinfection of adenovirus (ADV-3) to BCC-1/KMC cells. Different concentrations of caffeic acid [1 μg/ml (◆), 5 μg/ml (■), 20 μg/ml (▲), 40 μg/ml (X), 50 μg/ml (●)] were added at various time of preinfection (-2, -1 h), coinfection (0 h) or postinfection (1–24 h) of adenovirus (ADV-3) to BCC-1/KMC cells at 37 °C. After 3 days, optical density was evaluated by XTT method and expressed as the inhibition rate. The x-axis indicates the time course of adding caffeic acid. Each point represents the mean ± S.E. of triplicate samples of three independent experiments. The asterisk (*) indicates significant difference between test and DMSO control ($P < 0.01$).

4. Discussion

The present study has demonstrated that certain pure compounds of *P. major* possess antiviral activity in vitro. In contrast to results reported by McCutcheon et al. (1995), aqueous extract of *P. major* was found to possess a slight activity against HSV-2. The discrimination in results between the two studies might be due to the different strains of virus.

The water-soluble phenolic compounds (caffeic acid, chlorogenic acid, ferulic acid and *p*-coumaric acid) of *P. major* exhibited anti-HSV and ADV activity. The low antiviral activity of the aqueous extract of *P. major* may be explained by the low concentrations of these phenolic compounds present in the extract. Besides the slight direct antiviral activity of the aqueous extract of *P. major*, crude aqueous extract and 75% of the tested pure compounds possessed strong immunostimulating activity including secretion of interferon from human peripheral mononuclear cells in vitro (unpublished data). The property of direct antiviral effect, enhancing cellular immunity and secretion of interferon may explain the reason for the popular use of *P. major* in the traditional Chinese medicine for treating infectious diseases.

According to the recent reports on HSV-2, the EC_{50} of chlorogenic acid, caffeic acid and ferulic acid were 20, 54 and 8000 μg/ml, respectively (Neyts et al., 1992; Bourne et al., 1999). However, our results showed that ferulic acid ($\leqq 200$ μg/ml) possessed no activity whereas the EC_{50} of chlorogenic acid and caffeic acid were 86.5 and 87.3 μg/ml, respectively (Table 1). This discrepancy in results among the three different studies could be due to the difference in the strains of viruses, target cell and drug concentration used in the study.

A number of anti-HSV synthetic and plant drugs have been reported (Martin, 1987; Andersen et al., 1991; Ferrea et al., 1993; Wood, 1999; Bourne et al., 1999; Ikeda et al., 2000). The SI value of reference standard, acyclovir, is about 94. In the present study, the SI values of chlorogenic acid (83.9) was found to be as good as acyclovir, whilst caffeic acid was seven times better (SI value = 671) (Table 1).

Despite the great advances in the synthetic nucleoside analogues or cysteine protease inhibitors for anti-adenoviral replication, currently there is no approved chemotherapy in preventing or interrupting this viral infection (Dudgeon et al., 1969; Gordon et al., 1991; Kodama et al., 1996; Sircar et al., 1996; Mentel et al., 1997). New medications such as cidofovir, which is a broad-spectrum nucleoside monophosphate, appear to be effective against the adenoviruses in non-human systems and may have some effect in man (De Oliverira et al., 1996; Hedderwick et al., 1998; Ribaud et al., 1999). However, resistance of adenovirus to cidofovir treatment has also been reported to develop in vitro (Gordon et al., 1996).

Although 5-iodo-deoxyuridine has been clinically applied in treating adenoviral infection (Dudgeon et al., 1969), it was found to be quite toxic as its SI value was only 3.4 (Kodama et al., 1996). The safety of recent developed synthetic nucleoside analogues has improved dramatically, the SI values are generally greater than 290 (Kodama et al., 1996). A popular anti-adenovirus plant drug, ternatin, was reported to have a SI value of 20 (Simoes et al., 1990). Our study shows that four phenolic compounds, namely caffeic acid, chlorogenic acid, ferulic acid and *p*-coumaric acid, possess anti-adenoviral activity; the strongest anti-ADV-3 was caffeic acid with SI value 727, anti-ADV-8 and anti-ADV-11 were chlorogenic acid with SI values 36.9 and 301, respectively (Table 1). These findings suggests that the effect of these compounds on viruses is worth to be further investigated in vivo.

The present study has shown that caffeic acid possesses interesting anti-HSV-1, anti-HSV-2 and anti-ADV-3 activities and high SI values. In order to understand on how the caffeic acid inhibits the viral replication, a dose-dependent study and time course effect of this compound was investigated. Interestingly, caffeic acid was found to inhibit HSV-1 replication with an obvious dose-dependent manner, with EC_{50} 15.3 μg/ml and a 100% inhibition at concentration 40 μg/ml (Fig. 1).

Results on the time-course effect between 0 and 12 h after virus infection, caffeic acid was found to possess a similar trend of inhibition on viral replication. This suggests that the mode of action

is not derived from inhibiting the absorption of virus but resulting from viral replication after infection (Fig. 1). The time courses on the inhibition of ADV-3 and HSV-1 by caffeic acid were not similar, the major difference was that the inhibition of ADV-3 occurred during 0–2 h after infection whilst HSV-1 between 0 and 12 h, suggesting that caffeic acid inhibited ADV-3 at the early stage of replication. The discrepancy in the inhibition on the time-course effect between ADV-3 and HSV-1 by caffeic acid could be due to the difference in the unique family of viruses or different viral targets for the caffeic acid. The real antiviral mechanism remains to be further elucidated.

Among the five chemical classes tested, the phenolic compounds were showed to possess the major activities against human herpesviruses and adenoviruses infections. Compounds containing two hydroxyl groups at the R_1 and R_2 positions on the cinnamic acid moiety, namely caffeic acid and chlorogenic acid, exhibited a broader spectrum of antiviral activity than those possessing one hydroxyl group at the R_1 position such as ferulic acid and *p*-coumaric acid. Therefore, the spectrum of antiviral activity among the phenolic compounds containing the cinnamic acid moiety might be correlated to the number of hydroxyl group at the R_1 and R_2 positions.

The present study concludes that eleven pure compounds of the five different classes of chemicals found in extracts of *P. major* exhibited potent anti-HSV and/or ADV activities. Among them, the phenolic compounds, namely caffeic acid and chlorogenic acid, were found to possess the strongest antiviral activity. Due to the lack of approved drugs in treating adenoviral infection, caffeic acid and chlorogenic acid might be a potential therapeutic agent for treating this disease. As indicated by the high SI value ranging between 95 and 727, these two compounds are considered to be less toxic than the presently clinically used drug, 5-iodo-deoxyuridine (SI = 3.4). Therefore, the potential of caffeic acid and chlorogenic acid for use in treating adenoviral infection merit a greater attention.

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