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Anti-*Herpes Simplex* Virus Activity of *Bidens pilosa* and *Houttuynia cordata*

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Abstract: The present study evaluated the antiviral activity of *Bidens pilosa* L. var. minor (Blume) Sherff and *Houttuynia cordata* Thunb., using cytotoxicity test with XTT-based colorimetric assay. BCC-1/KMC cells were infected with *herpes simplex* virus (HSV) and then were cultured with hot water extract of *B. pilosa* (HWBP) or *H. cordata* (HWHC). Results showed that HWBP significantly inhibited the replication of HSV at a concentration of 100 μg/ml (11.9% for HSV-1, $p < 0.01$; 19.2% for HSV-2, $p < 0.005$), whereas HWHC had the same effect at a concentration of 250 μg/ml (10.2% for HSV-1, $p < 0.05$; 32.9% for HSV-2, $p < 0.005$). The ED_{50} of HSV type 1 (HSV-1) and HSV type 2 (HSV-2) for HWBP was 655.4 μg/ml and 960 μg/ml respectively, for HWHC it was 822.4 μg/ml and 362.5 μg/ml respectively. Both drugs had selective indexes above 1.04. *H. cordata* had better effect against HSV-2 than HSV-1, and had a low ED_{50} against HSV-2. We suggest that *H. cordata* might be a useful medicinal plant against infection of HSV-2.

Keywords: *Bidens pilosa*; *Houttuynia cordata*; *Herpes Simplex* Virus.

Introduction

During the past 50 years, a great deal of interest had been initiated in screening therapeutic agents from plants. *Bidens pilosa* L. was widely used as a traditional remedy for treating influenza, diabetes and gastroenteritis worldwide. *Houttuynia cordata* Thunb. was used in traditional Chinese medicine for treating infectious disease, refractory hemoptysis, malignant pleural effusion, nephrotic syndrome (Zheng *et al.*, 1998). Studies had shown that *B. pilosa* possessed antihyperglycemic (Ubillas *et al.*, 2000), antiulcerogenic (Alvarez *et al.*, 1999),

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immunosuppressive (Pereira *et al.*, 1999), anti-inflammatory (Pereira *et al.*, 1999; Jager *et al.*, 1996; Chih *et al.*, 1995; Geissberger and Sequin, 1991), vasodilative (Dimo *et al.*, 1998), antimalarial (Brandao *et al.*, 1997), antibacterial (Geissberger and Sequin, 1991; Rabe and van Staden, 1997), and hepatoprotective activities (Chin *et al.*, 1996). However, there was no previous report of antiviral activity. Also co-carcinogenesis (Mirvish *et al.*, 1979; Mirvish *et al.*, 1985) and photocytotoxicity (Arnason *et al.*, 1980; Wat *et al.*, 1979) were reported with different species of *B. pilosa*.

H. cordata was reported to possess antimicrobial (Zheng *et al.*, 1998), antiviral (Zheng *et al.*, 1998; Hayashi *et al.*, 1995), immunostimulatory (Zheng *et al.*, 1998), diuretic (Zheng *et al.*, 1998), anticancer (Zheng *et al.*, 1998), sedative (Zheng *et al.*, 1998), anti-inflammatory (Zheng *et al.*, 1998) and antitussive effects (Zheng *et al.*, 1998). Although *H. cordata* was found to have activity against *herpes simplex* virus type 1 (HSV-1) (Hayashi *et al.*, 1995), no effect against *herpes simplex* virus type 2 (HSV-2) was reported (Zheng *et al*., 1998). In searching natural crude drugs for potential anti-HSV activity, hot water extracts of whole plant of *B. pilosa* (HWBP) and *H. cordata* (HWHC) were tested.

Materials and Methods

Preparation of Tested Drugs

Hot water extracts of *B. pilosa* (HWBP) and *H. cordata* (HWHC) were prepared as follows: 100 g of dried whole plant of *B. pilosa* or *H. cordata* were added with 1000 ml reverse-osmotic water in a flask. They were boiled for 1 hour and then supernatant were collected. Three repetitions of these procedures were done. All these supernatants were mixed for filtration by a filter and for dryness in a vacuum. Dissolution and dilution of the powder with di-distilled water to concentrations of 100 µg/ml, 250 µg/ml and 500 µg/ml were done before experiments (Chang and Yeung, 1988). Acyclovir and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (USA). Acyclovir was suspended in DMSO and diluted with di-distilled water to concentrations of 0.1 µg/ml, 0.5 µg/ml and 1 µg/ml before use.

Cells

BCC-1/KMC cell line (Chiang *et al.*, 1994) was used for viral culture and antiviral screening. Cells were cultured with RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotics (10000 u/ml penicillin, 10 mg/ml streptomycin, 25 µg/ml amphotericin B; Sigma, USA) at 37°C in a humidified atmosphere containing 5% CO_2.

Cytoprotective Activity Against HSV with XTT-based Colorimetric Assay

BCC-1/KMC cells, treated by trypsin, were seeded into 96-well plates with a concentration of 1×10^5 cells/ml and a volume of 70 µl per well. After incubation for 6 hours at 37°C with 5% CO_2, 20 µl HSV-1 containing 25 $TCID_{50}$ or 20 µl HSV-2 containing 20 $TCID_{50}$ was

added. The mixtures were incubated for another 2 hours, followed by adding different concentrations of hot water extracts to the triplicate culture wells. DMSO was used as a negative control while acyclovir solution was used as a positive control. After incubation at 37°C with 5% CO_2 for three days, a mixture of 0.l ml PMS (electron-coupling reagent) and 5 ml XTT (Sigma, USA) was added to each well in a volume of 50 µl. The trays were re-incubated for another 2 hours to allow XTT formazan production. The content of each plate was mixed and the optical density was determined with the ELISA reader (Multiskan EX, Labsystems) at 450 nm as test wavelength and 690 nm as reference wavelength.

Cytoprotection rate was calculated as $(OD_{tv}–OD_{cv})/(OD_{cd}–OD_{cv}) \times 100\%$. OD_{tv} represents the absorbance of the test compounds with virus-infected cells. OD_{cv} represents the absorbance of viral control. OD_{cd} was the absorbance of the cell control only. The antiviral dose of 50% effectiveness (ED_{50}) was defined as the concentration which achieved 50% cytoprotection against viral infection. The concentration of 50% cytotoxicity (CC_{50}) of normal human lymphocytes (3×10^6 Cells/ml) was assayed and calculated by the above methods. The selective index was determined by the ratio of CC_{50} to ED_{50}.

Statistical Analysis

Means and standard errors were calculated with the Excel software for Windows. Chi-square test with Yate's correction was used to calculate p value between control and samples by the SPSS Base 8.0 software for Windows. Difference with $p < 0.05$ was considered as statistically significant.

Results

Both HWBP and HWHC showed a significant inhibitory activity against infection of HSV (Tables 1 and 2). The inhibitory effects were dose-dependent (Figs. 1 and 2). At a concentration of 100 µg/ml HWBP, 11.9% ($p < 0.01$) of cells were protected from infection of HSV-1 and 19.2% ($p < 0.005$) from infection of HSV-2 (Fig. 1 and Table 1). In the case of HWHC, at a concentration of 250 µg/ml, 10.2% ($p < 0.05$) of cells were protected from infection of HSV-1 and 32.9% ($p < 0.005$) from infection of HSV-2 (Fig. 2 and Table 2). The inhibitory effect of HWHC against HSV-2 was comparable to that of acyclovir (0.5 µg/ml) and was better than that against HSV-1.

Table 1. Cytoprotection Rates of *B. pilosa* Against HSV Infection

	HWBP (µg/ml)				
	Solvent Control	**100**	**250**	**500**	**Acyclovir 0.5**
HSV-1 (%)	1.52	11.92*	23.82†	39.02†	45.07
HSV-2 (%)	0.26	19.21†	26.72†	33.07†	33.29

Data represented an average of three tests.

*$p < 0.01$; †$p < 0.005$ (chi-square test with Yate's correction).

Table 2. Cytoprotection Rates of *H. cordata* Against HSV Infection

	HWHC (μg/ml)				
	Solvent Control	**100**	**250**	**500**	**Acyclovir 0.5**
HSV-1 (%)	1.52	4.71	10.23*	27.6†	45.07
HSV-2 (%)	0.26	5.37	32.86†	70.96†	33.29

Data represented an average of three tests.
*p < 0.05; †p < 0.005 (chi-square test with Yate's correction).

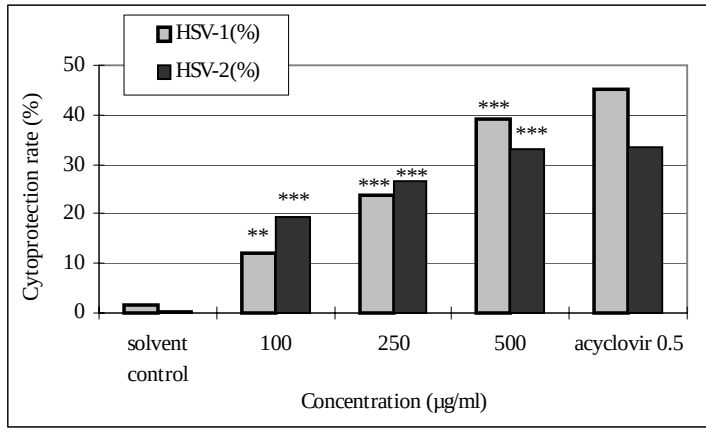

Figure 1. Cytoprotection rate of *B. pilosa* against HSV infection. Solvent was used as negative control. Acyclovir (0.5 μg/ml) was used as positive control (**p < 0.01; ***p < 0.005).

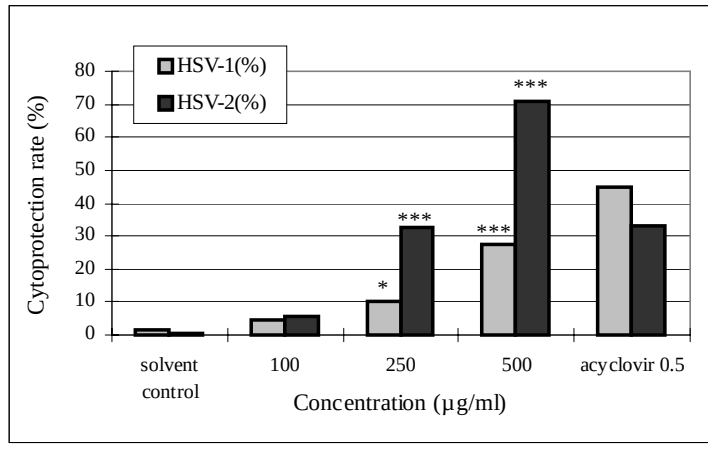

Figure 2. Cytoprotection rate of *H. cordata* against HSV infection. Solvent was used as negative control. Acyclovir (0.5 μg/ml) was used as positive control (*p < 0.05; ***p < 0.005).

HWBP had a better effect than that of HWHC in protecting cells from infection by HSV-1 ($p < 0.025$, Fig. 3 and Table 3) at concentration of 250 µg/ml. The same result was also found in preventing infection from HSV-2 ($p < 0.01$, Fig. 4 and Table 3) at a lower concentration (100 µg/ml). However, HWBP exhibited less effect than that of HWHC in protecting cells from infection of HSV-2 ($p < 0.005$, Fig. 4 and Table 3) at a higher concentration (500 µg/ml). The cytoprotection rate of HWHC against infection of HSV-2 rapidly increased as the concentration rose (5.4% at a concentration of 100 µg/ml to 71.0% at a concentration of 500 µg/ml, Table 2). The ED_{50} of HWHC was 362.5 µg/ml (Table 4).

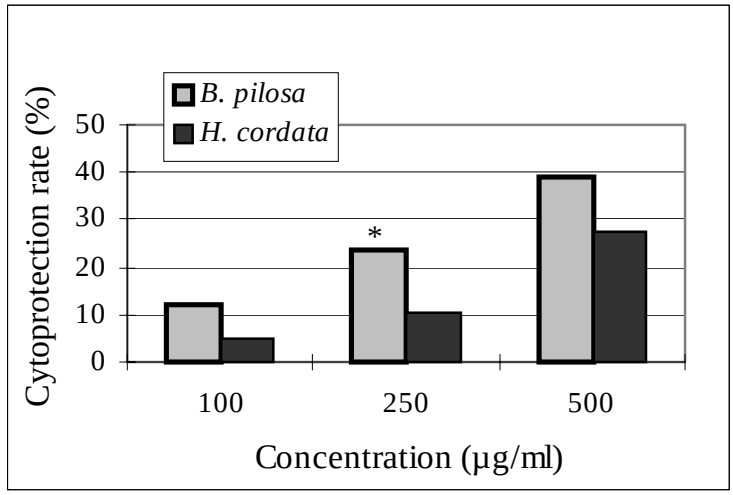

Figure 3. Comparison of cytoprotection rate between *B. pilosa* and *H. cordata* against HSV-1 infection. *B. pilosa* had better effect than that of *H. cordata* at a concentration of 250 µg/ml (*$p < 0.025$). There was no difference between other concentrations ($p > 0.05$).

Table 3. Cytoprotection Rates of *B. pilosa* and *H. cordata* Against HSV Infection

		HWBP and HWHC (µg/ml)		
		100	**250**	**500**
HSV-1 (%)	***B. pilosa***	11.92	23.82*	39.02
	H. cordata	4.71	10.23	27.6
HSV-2 (%)	***B. pilosa***	19.21†	26.72	33.07
	H. cordata	5.37	32.86	70.96‡

Data represented an average of three tests.

*$p < 0.025$; †$p < 0.01$; ‡$p < 0.005$ (chi-square test with Yate's correction).

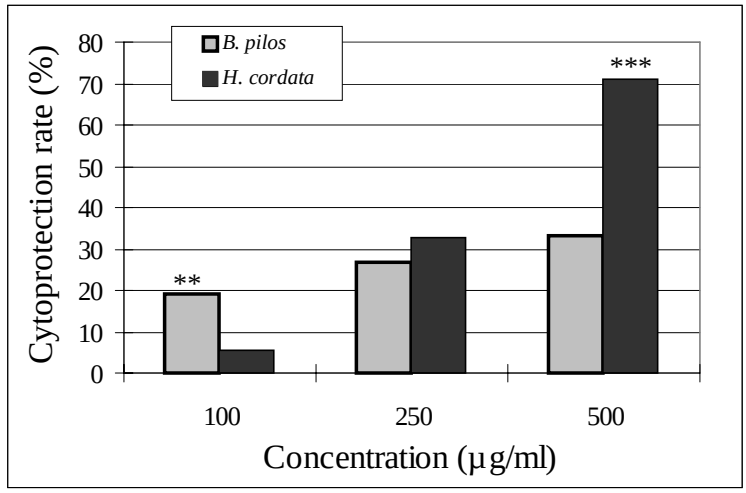

Figure 4. Comparison of cytoprotection rate between *B. pilosa* and *H. cordata* against HSV-2 infection. The effect of *H. cordata* had rapidly increased when drug concentration rose (**p < 0.01; ***p < 0.005).

Table 4. Antiviral Activity of *B. pilosa* and *H. cordata*

	B. pilosa		***H. cordata***		**Acyclovir**	
	ED_{50} (µg/ml)	**SI**	**ED_{50} (µg/ml)**	**SI**	**ED_{50} (µg/ml)**	**SI**
HSV-1	655.44	>1.53	822.39	>1.22	0.61	17.26
HSV-2	960	>1.04	362.46	>2.76	0.81	12.9

ED_{50}: Concentration of 50% cytoprotection.

SI: Selective index = CC_{50}/ED_{50}.

CC_{50}: Concentration of 50% cytotoxicity. CC_{50} of *B. pilosa* and *H. cordata* were > 1000 µg/ml. CC_{50} of acyclovir was 10.44 µg/ml.

Discussion

Much effort had been made to search for new antiviral therapeutic agents during the past 50 years. This had led to numerous studies of medicinal plants for clinical applications (Carter and Livingston, 1976; Marsoni and Wittes, 1984). *B. pilosa* and *H. cordata* had been widely used in the traditional medicine of different ethnic groups in the world. *H. cordata* was reported to have activity against HSV-1 (Hayashi *et al.*,1995), but not HSV-2 (Zheng *et al.*, 1998). In this study, we demonstrated that both HWBP and HWHC possessed antiviral activity and the activity was dose-dependent (Figs. 1 and 2). In contrast to the findings of Zheng *et al.* (1998), *H. cordata* did have activity against HSV-1 and HSV-2. The activity against HSV-2 was better than that against HSV-1 (Fig. 2 and Table 2).

The ED_{50} of *B. pilosa* and that of *H. cordata* were much higher than ED_{50} of acyclovir (Table 4). We still believed that both plants had value for clinical application. Several reasons could explain the higher ED_{50} of *B. pilosa* and *H. cordata*. The first, concentration of active

compounds of the plant materials might be too low or active components might be reduced during extraction. The second, they might become more active after transformation *in vivo* (Marsoni and Wittes, 1984; Double, 1992). Therefore, purification of the effective components from these crude drugs might be necessary.

High CC_{50} (>1000 µg/ml) of HWBP and HWHC indicated a good tolerability by human cells. It will be possible to use these plants against infection of HSV in the future (Table 4).

According to our results, it is worthy to study *B. pilosa* and *H. cordata* further. The fractionation, separation of active components and clarification of their mechanism of action are currently under investigation.

Conclusion

Hot water extracts of *B. pilosa* and *H. cordata* showed activity against HSV. The present study has demonstrated the potential clinical implication of *B. pilosa* and *H. cordata*. Hence, they are worthy of further investigation.

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