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## Antileukemic Activity of *Bidens pilosa* L. var. *minor* (Blume) Sherff and *Houttuynia cordata* Thunb.

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**Abstract:** To evaluate the anti-leukemic activity of *Bidens pilosa* L. var. *minor* (Blume) Sherff and *Houttuynia cordata* Thunb., cytotoxicity tests with an XTT-based colorimetric assay were used. Five leukemic cell lines, namely L1210, U937, K562, Raji and P3HR1, were cultured with hot water extracts of *B. pilosa* var. *minor* or *H. cordata*. Hot water extracts of *B. pilosa* var. *minor* inhibited these five leukemic cells with IC<sub>50</sub>s between 145 µg/ml and 586 µg/ml. The effect was greatest on four cell lines, namely L1210, P3HR1, Raji and K562, with IC<sub>50</sub>s below 200 µg/ml and a selective index of more than 5. Hot water extract of *H. cordata* inhibited these five leukemic cells with IC<sub>50</sub>s between 478 µg/ml and 662 µg/ml. The selective index was between 1.5 and 2.1. *B. pilosa* var. *minor* was more effective than *H. cordata* in inhibiting most of the leukemic cells in our study. We suggest that *B. pilosa* L. var. *minor* (Blume) Sherff may prove to be a useful medicinal plant for treating leukemia.

During the past fifty years, there had been a great deal of interest in screening therapeutic agents from plants. *Bidens pilosa* L. is widely used in traditional medicine for anti-influenza, diabetic control and treatment of gastroenteritis. *Houttuynia cordata* Thunb. is used in traditional Chinese medicine for treating infectious disease, refractory hemoptysis, malignant pleural effusion and nephrotic syndromes (Zheng *et al.*, 1998). Studies have shown that *B. pilosa* possessed anti-hyperglycemic (Ubillas *et al.*, 2000), Anti-ulcerogenic (Alvarez *et al.*, 1999), immuno-suppressive (Pereira *et al.*, 1999), anti-inflammatory (Pereira *et al.*, 1999; Jager *et al.*, 1996; Chih *et al.*, 1995; Geissberger and Sequin, 1991), vaso-dilative (Dimo *et al.*, 1998), hypotensive (Dimo *et al.*, 1999), anti-malarial (Brandao *et al.*, 1997), anti-bacterial (Geissberger and Sequin, 1991; Rabe and van Staden, 1997) and

hepato-protective activities (Chin *et al.*, 1996). But also co-carcinogenesis (Mirvish *et al.*, 1985; Mirvish *et al.*, 1979) and photo-cytotoxicity (Arnason *et al.*, 1980; Wat *et al.*, 1979) were reported with different species of *B. pilosa*. *H. cordata* has biologic activities including anti-microbial (Zheng *et al.*, 1998), anti-viral (Zheng *et al.*, 1998; Hayashi *et al.*, 1995) immunostimulatory (Zheng *et al.*, 1998), diuretic (Zheng *et al.*, 1998), anti-cancer (Zheng *et al.*, 1998), sedative (Zheng *et al.*, 1998), anti-inflammatory (Zheng *et al.*, 1998) and anti-tussive effects (Zheng *et al.*, 1998). Although *H. cordata* was reported to be active against ascitic tumor (Zheng *et al.*, 1998), it was unknown whether hot water extracts (HW) of *B. pilosa* var. *minor* or *H. cordata* had anti-leukemic activity. In searching natural crude drugs for potential anti-leukemic activity, we tested whole plant HW extracts of *B. pilosa* var. *minor* and *H. cordata*.

## Materials and Methods

### *Preparation of Tested Drugs*

Hot water (HW) extracts of *B. pilosa* var. *minor* and *H. cordata* were prepared from the whole plant according to the standard methods with minor modification as described earlier by Chang and Yeung (1988). 5-Fluorouracil (5-FU) and DMSO were purchased from Sigma Chemical Co. (USA). 5-FU was suspended in DMSO and diluted with distilled water at concentrations as indicated in the tables before use.

### *Cells*

Five leukemic cell lines, namely U937 (ATCC CRL 1593), K562 (ATCC CCL 243), Raji (ATCC CCL 86), P3HR1 (ATCC HTB62) and L1210 (ATCC CCL219) were used for evaluation of anti-leukemic activity. They were cultured with RPMI 1640 supplemented with 10% fetal bovine serum and 1% antibiotics (final concentrations were 100 u/ml penicillin G sodium, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin sulfate) (Sigma, USA) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### *Cytotoxicity Test of Leukemic Cell with XTT-based Colorimetric Assay*

All leukemic cells were seeded into 96-well plates (Corning Co. USA) with a concentration of  $1 \times 10^5$  cells/ml and a volume of 90 µl per well. Different concentrations of HW-extract were applied to triplicate culture wells. 0.05% (w/w) DMSO was used as negative control and several concentrations of 5-FU were used as positive control. After incubation at 37°C with 5% CO<sub>2</sub> for three days, 50 µl of a mixture of 0.1 ml PMS (electron-coupling reagent) and 5 ml XTT (Sigma, USA) was added to each well. The content of each well was mixed and the trays were incubated for another 2 hrs to allow XTT formazan production. The optical density was determined with the ELISA reader (Multiskan EX, Labsystems) at a test wave-length of 450 nm and a reference wave-length of 690 nm.

Data was calculated as percentage of inhibition by the following formula: inhibition rate (%) =  $\{100 - (\text{ODt}/\text{ODm} \times 100)\}$ %. ODt and ODm indicated the absorbance of the test compounds and the medium control respectively. The concentration of 50% inhibition (IC<sub>50</sub>) was defined as the concentration that achieved 50% cytotoxicity against cancer cell. The concentration of 50% cytotoxicity (CC<sub>50</sub>) of normal human lymphocytes ( $3 \times 10^6$

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cells/ml) was assayed and calculated by the above methods. The selective index was determined by the ratio of the  $CC_{50}$  to the  $IC_{50}$ .

### Statistical Analysis

Means and standard errors were calculated with software of Excel for Windows. The Chi-square test with Yate's correction was used to calculate P values between control and experimental samples (SPSS Base 8.0 for Windows). Difference with a P value less than 0.05 was considered statistically significant.

### Results

HW-extracts of *B. pilosa var. minor* and *H. cordata* showed activity against leukemic cells as compared with solvent control. The effect was dose-dependent (Figures 1 and 2, Tables 1 and 2). HW-extracts of *B. pilosa var. minor* inhibited L1210 cells (22.04%,  $P < 0.01$ , Figure 3), P3HR1 cells (15.91%,  $P < 0.005$ , Figure 4), Raji cells (39.29%,  $P < 0.005$ , Figure 5), K562 cells (31.07%,  $P < 0.005$ , Figure 6) at a concentration of 100  $\mu\text{g/ml}$ . U937 cells were inhibited (6.81%,  $P < 0.05$ , Figure 7) at a concentration of 250  $\mu\text{g/ml}$ . HW-extract of *H. cordata* inhibited L1210 cells (17.95%,  $P < 0.01$ , Figure 3), P3HR1 cells (11.73%,  $P < 0.025$ , Figure 4), Raji cells (15.63%,  $P < 0.005$ , Figure 5), and U937 cells (21.48%,  $P < 0.005$ , Figure 7) at a concentration of 100  $\mu\text{g/ml}$ . K562 cells were inhibited (18.23%,  $P < 0.005$ , Figure 6) at a concentration of 250  $\mu\text{g/ml}$ .

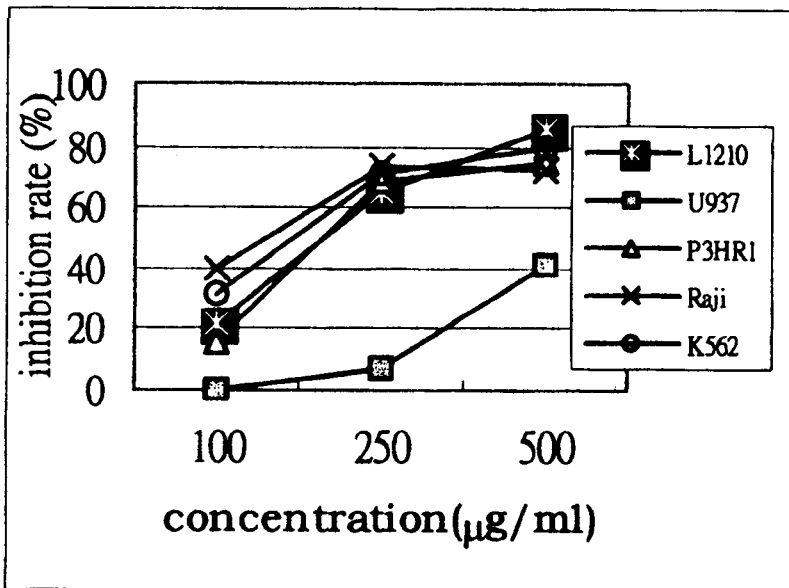


Figure 1. Antileukemic activity of *B. pilosa var. minor*. The activity was dose-dependent. All cells except U937 cell showed significant inhibition at a concentration of 100  $\mu\text{g/ml}$  ( $P < 0.01$ ). The U937 cell line was inhibited at a concentration of 250  $\mu\text{g/ml}$  ( $P < 0.05$ ). Each point represents an average of 9 tests.

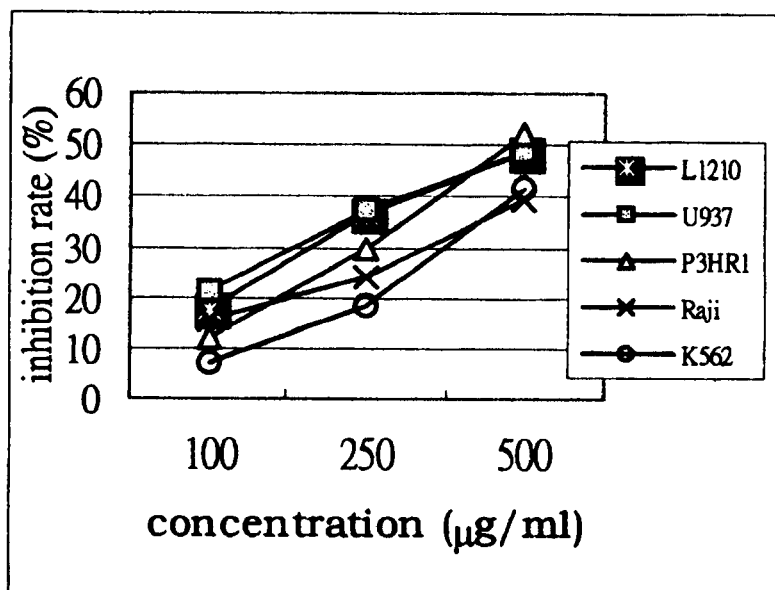


Figure 2. Antileukemic activity of *H. cordata*. The activity was dose-dependent. All cells except K562 cell showed significant inhibition at a concentration of 100 µg/ml ( $P < 0.025$ ). The K562 cell line was inhibited at a concentration of 250 µg/ml ( $P < 0.005$ ). Each point represents an average of 3 tests.

**Table 1. Inhibition Rates of *B. pilosa var. minor* against Different Leukemic Cells**

		(µg/ml)			
	solvent control	100	250	500	5FU#
L1210 (%)	5.2	22.04**	65.1***	85.28***	78.63
U937 (%)	0.45	0	6.81*	41.2***	41.88
P3HR1 (%)	1.76	15.91***	68.45***	74.48***	54.64
Raji (%)	0.2	39.29***	74.08***	72.21***	59.57
K562 (%)	0.94	31.07***	70.74***	79.86***	53.28

Each inhibition rate represents average of 9 tests

\*  $P < 0.05$ . \*\*  $P < 0.01$ . \*\*\*  $P < 0.005$  (compared with solvent control)

# 5FU was used as positive control at a concentration of 0.1 µg/ml for L1210 cells;

0.4 µg/ml for U937 cells; 1 µg/ml for P3HR1 and Raji cells; 0.5 µg/ml for K562 cells.

HW-extract of *B. pilosa var. minor* had a stronger effect than *H. cordata* in inhibiting Raji cells (39.29% vs 15.63%,  $P < 0.005$ , Table 3) and K562 cells (31.07% vs 6.95%,  $P < 0.005$ , Table 3) at a concentration of 100 µg/ml. A similar effect was found in inhibiting L1210 cells (65.1% vs 36.41%,  $P < 0.005$ , Table 3) and P3HR1 cells (68.45% vs 29.62%,  $P < 0.005$ , Table 3) at a concentration of 250 µg/ml. But HW-extract of *B. pilosa var. minor*

**Table 2. Inhibition Rates of *H. cordata* against Different Leukemic Cells**

	(µg/ml)				5FU#
	solvent control	100	250	500	
L1210 (%)	5.2	17.95**	36.41***	48.71***	78.63
U937 (%)	0.45	21.48***	37.29***	48.67***	41.88
P3HR1 (%)	1.76	11.73*	29.62***	51.92***	54.64
Raji (%)	0.2	15.63***	24.11***	39.82***	59.57
K562 (%)	0.94	6.95	18.23***	41.36***	53.28

Each inhibition rate represents average of 3 tests

\* P < 0.05. \*\* P < 0.01. \*\*\* P < 0.005 (compared with solvent control).

# 5FU was used as positive control at a concentration of 0.1 µg/ml for L1210 cells;

0.4 µg/ml for 0937 cells; 1 µg/ml for P3HR1 and Raji cells; 0.5 µg/ml for K562 cells.

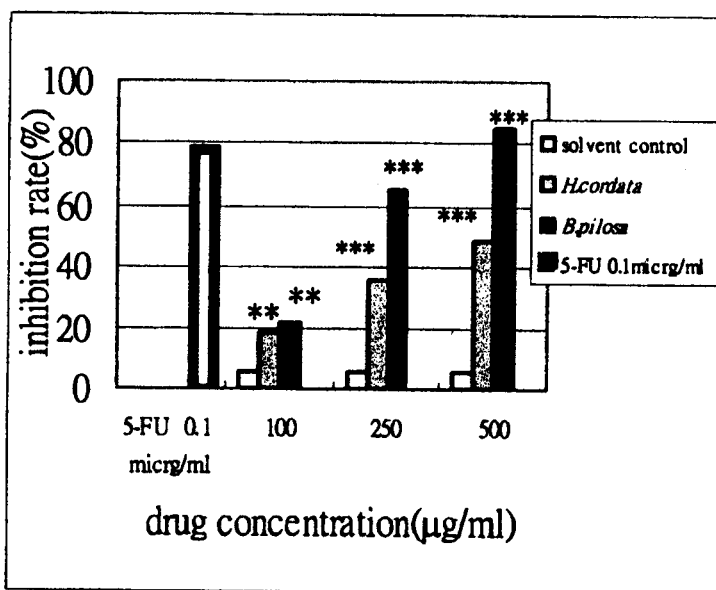


Figure 3. Antileukemic activity of tested drugs against L1210 cells. Solvent was used as negative control. 5-FU (0.1 µg/ml) was used as positive control. *B. pilosa* var. *minor* and *H. cordata* showed significant inhibitory ability at a concentration of 100 µg/ml (P<0.01). (\*\* P<0.01; \*\*\* P<0.005).

had less effect than *H. cordata* in inhibiting U937 cells (0% vs 21.48%, P<0.005, Table 3) at a concentration of 100 µg/ml.

## Discussion

Since the end of the 1950s, a great deal of effort had been made in searching for new therapeutic agents. This led to numerous studies of medicinal plants for clinical applications

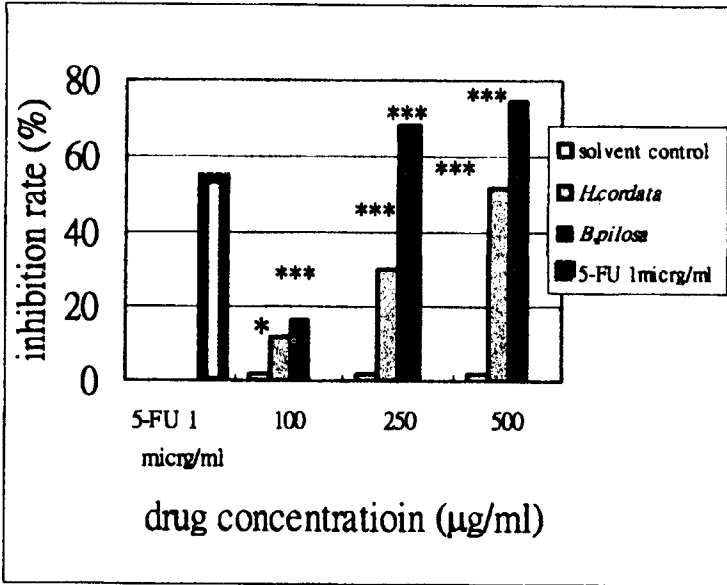


Figure 4. Antileukemic activity of tested drugs against P3HR1 cells. Solvent was used as negative control. 5-FU (1 µg/ml) was used as positive control. *B. pilosa var. minor* and *H. cordata* showed significant inhibitory ability at a concentration of 100 µg/ml ( $P < 0.025$ ). (\*  $P < 0.025$ ; \*\*\*  $P < 0.005$ ).

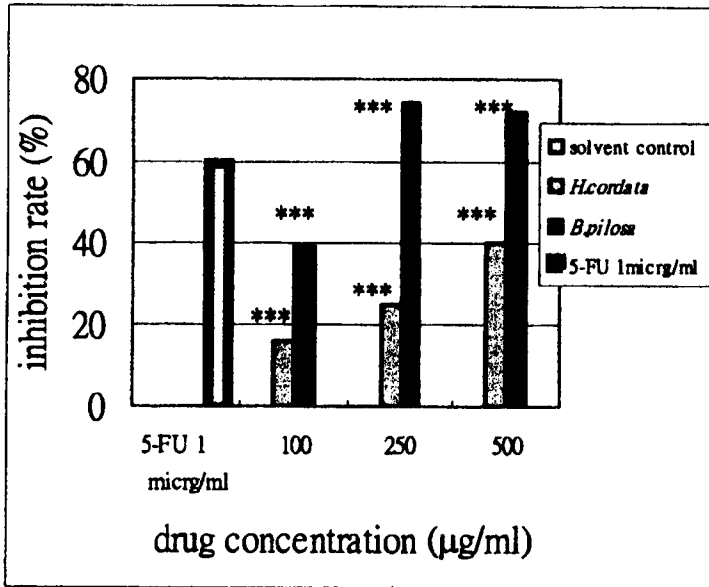


Figure 5. Antileukemic activity of tested drugs against Raji cells. Solvent was used as negative control. 5-FU (1 µg/ml) was used as positive control. *B. pilosa var. minor* and *H. cordata* showed significant inhibitory ability at a concentration of 100 µg/ml ( $P < 0.005$ ) (\*\* $P < 0.005$ ).

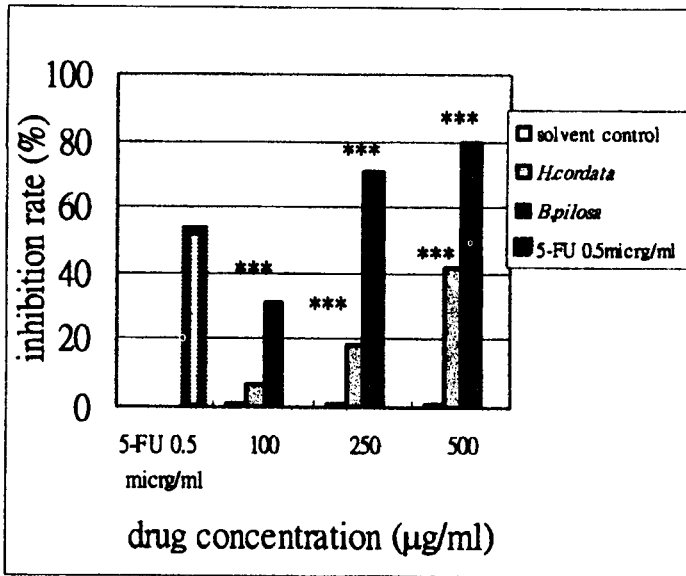


Figure 6. Antileukemic activity of tested drugs against K562 cells. Solvent was used as negative control. 5-FU (0.5 µg/ml) was used as positive control. *B. pilosa* var. *minor* showed significant inhibitory ability at a concentration of 100 µg/ml ( $P < 0.005$ ). *H. cordata* showed significant inhibitory ability at a concentration of 250 µg/ml ( $P < 0.005$ ) (\*\*\*) ( $P < 0.005$ ).

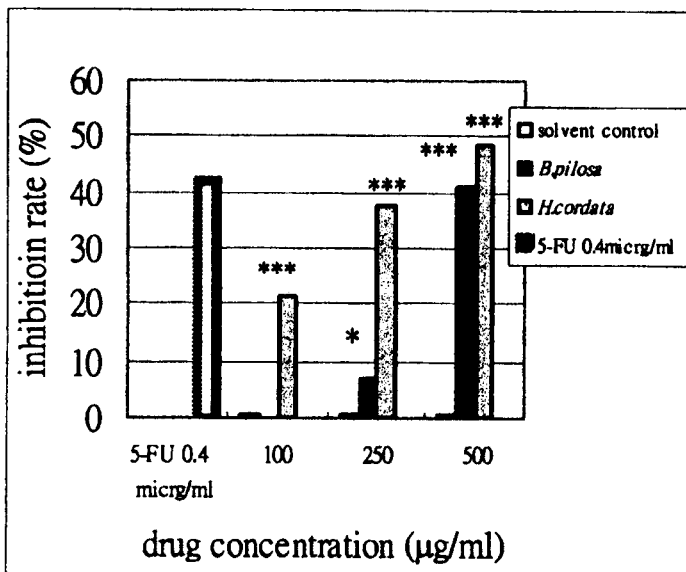


Figure 7. Antileukemic activity of tested drugs against U937 cells. Solvent was used as negative control. 5-FU (0.4 µg/ml) was used as positive control. *H. cordata* showed significant inhibitory ability at a concentration of 100 µg/ml ( $P < 0.005$ ). *B. pilosa* var. *minor* showed significant inhibitory ability at a concentration of 250 µg/ml ( $P < 0.05$ ) (\*  $P < 0.05$ ; \*\*\*  $P < 0.005$ ).



**Table 3. Inhibition Rates of *H. cordata* and *B. pilosa var. minor* against Different Leukemic Cells**

		(µg/ml)		
		100	250	500
L1210 (%)	<i>B. pilosa</i>	22.4	65.1***	85.28***
	<i>H. cordata</i>	17.95	36.41	48.71
U937 (%)	<i>B. pilosa</i>	0	6.81	41.2
	<i>H. cordata</i>	21.48***	37.29***	48.67
P3HR1 (%)	<i>B. pilosa</i>	15.91	68.45***	74.48***
	<i>H. cordata</i>	11.73	29.62	51.92
Raji (%)	<i>B. pilosa</i>	39.29***	74.08***	72.21***
	<i>H. cordata</i>	15.63	24.11	39.82
K562 (%)	<i>B. pilosa</i>	31.07***	70.74***	79.86***
	<i>H. cordata</i>	6.95	18.23	41.36

\*\*\* P &lt; 0.005

**Table 4. Antileukemic Activities of *B. pilosa var. minor* and *H. cordata* against Different Leukemic Cells**

	<i>B. pilosa var. minor</i>		<i>H. cordata</i>		5-FU	
	IC <sub>50</sub> (µg/ml)	SI	IC <sub>50</sub> (µg/ml)	SI	IC <sub>50</sub> (µg/ml)	SI
L1210	197.3	>5.07	526.2	>1.9	0.08	>125
U937	586.52	>1.71	529.22	>1.89	0.45	>22.2
P3HR1	196.46	>5.09	478.48	>2.09	0.96	>10.4
Raji	145.74	>6.86	662	>1.51	0.92	>10.9
K562	171.46	>5.83	593.39	>1.69	0.47	>21.3

IC<sub>50</sub>: Concentration of 50% inhibition.CC<sub>50</sub>: Concentration of 50% cytotoxicity. *B. pilosa* > 1000 mg/ml. *H. cordata* > 1000 mg/ml. 5-FU > 10 mg/ml.SI: Selective index = CC<sub>50</sub>/IC<sub>50</sub>

(Carter and Livingston, 1976; Marsoni and Wittes, 1984). *B. pilosa var. minor* and *H. cordata* have been widely used in traditional medicine. Anti-leukemic effects have never been reported before. Our results suggest both plants might have anti-leukemic activity at a concentration of 250 µg/ml (Tables 1 and 2). The activity was dose-dependent (Figures 1 and 2).

Although the IC<sub>50</sub> of *B. pilosa var. minor* and *H. cordata* were much higher than that of 5-FU (Table 4), this does not mean that both plants have little value for clinical application. Some effective components might be lost during extraction or they might need to be metabolized *in vivo* to become more active (Marsoni and Wittes, 1984; Double, 1992). Therefore, further purification of effective components from these crude drugs is necessary.

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High  $CC_{50}$  ( $>1000$   $\mu\text{g/ml}$ ) of HW-extracts of *B. pilosa* var. *minor* and of *H. cordata* indicate good tolerability by human cells. This might make them useful in treating human leukemia in the future (Table 4).

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

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