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# *In vitro* Anti-leukemic and Antiviral Activities of Traditionally Used Medicinal Plants in Taiwan

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Abstract: Medicinal plants have been historically used as treatment for different kinds of human diseases. In this study, hot water (HW) extract of five Taiwanese traditionally used medicinal plants was evaluated for their *in vitro* anti-leukemic (including anti-K562, L1210, P3HR1, Raji and U937 leukemia cells) and antiviral (including HSV-1 and HSV-2) activities. Results showed that *Blumea lacera* exhibited broad anti-leukemic activity at magnitudes ranging from moderate to mild and *Ixeris chinensis* is effective at inhibiting the proliferation of K562 cells. *B. lacera* and *Tithonia diversifolia* suppressed the replication of HSV-1 and HSV-2, and had $IC_{50}$ values below 100 μg/ml. The medicinal plants showed no cytotoxic effect at concentrations that inhibited HSV infection. It was, therefore, concluded that the HW extract of tested medicinal plants exhibited anti-leukemic and antiviral activities at different magnitudes of potency.

*Keywords*: Antileukemic Activity; Antiviral Activity; *Blumea lacera*; *Ixeris chinensis*; *Tithonia diversifolia*.

## Introduction

Cancer and infectious disease are two main causes of morbidity and mortality in modern life. Since the 20th century, many antibiotics and semi-synthetic analogues have been successfully developed, and clinically used as treatment for cancer and virus infections.

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However, the results are not quite always satisfactory. The licensed medicines are often unsuitable or ineffective in treatment because of their side effects and the appearance of resistance mutant problems (Coen, 1994; Garattini and La Vecchia, 2001). Therefore, a continuous search for new anticancer and antiviral agents is desired.

Medicinal plants have been historically used as treatment for various ailments, including cancer and virus infections. During the past 30 years, several extracts and pure compounds from plants are reported to exhibit anti-cancer and anti-viral activities in literature (Carter and Livingston, 1976; Becker, 1980; Parkinson *et al.*, 1994; Abad *et al.*, 2000). According to Cragg's report, approximately 60% of antitumor and anti-infective agents that are commercially available or in the late stages of clinical trials today were of natural origin (Cragg *et al.*, 1997). These observations suggested that medicinal plants could be applied as alternative in searching novel anticancer and antiviral agents.

In this study, hot water (HW) extract of five Taiwanese traditionally used medicinal plants, *Blumea lacera* (Burn. f.) DC. (Compositae), *Eclipta prostrata* Linn. (Compositae), *Ixeris chinensis* (Thunb.) Nakai (Compositae), *Senecio scandens* Buch.-Ham. ex D. Don (Compositae) and *Tithonia diversifolia* (Hewsl.) A. Gray (Compositae), were investigated for their *in vitro* anti-leukemic and antiviral activities. These medicinal plants are previously found to possess many biological activities. For example, they can enhance murine immunity system (He *et al.*, 1992) and neutralize the South American rattlesnake's venom (Mors *et al.*, 1989). They also exhibit hepatoprotective (Chiu *et al.*, 1989; Lin *et al.*, 1994), cytotoxic (Wu *et al.*, 2001; Gu *et al.*, 2002), anti-diabetic (Miura *et al.*, 2002), anti-HIV-1 (Cos *et al.*, 2002), anti-inflammatory (Rungeler *et al.*, 1998), anti-peroxidation (Liu and Ng, 2000), anti-plasmodial (Goffin *et al.*, 2002), anti-ameobic, antibacterial and spasmolytic activities (Tona *et al.*, 1999 and 2000). It was, however, unknown whether or not these plants possessed anti-leukemic and anti-herpes simplex virus (HSV) activities. This is the first report of the related medicinal plants on the anti-leukemic and anti-HSV activities. HW extract of *Bidens pilosa* L. *var. minor* (Blume) Sherff (Compositae), which has been reported to exhibit anti-leukemic (Chang *et al.*, 2001) and anti-HSV (Chiang *et al.*, 2003) activities, was used as a positive control.

## Materials and Methods

### *Plant Materials*

Whole plant of *B. lacera*, *B. pilosa*, *E. prostrata*, *I. chinensis*, *S. scandens* and *T. diversifolia* were collected from southern Taiwan. Authenticity was identified and confirmed using morphological and anatomical analysis by Professor Chun-Ching Lin, Graduate Institute of Pharmaceutical Sciences, College of Pharmacy, Kaohsiung Medical University, Taiwan. Voucher specimens are deposited at the Herbarium of the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

*Preparation of HW Extracts*

HW extracts from the dried whole plant of *B. lacera*, *B. pilosa*, *E. prostrata*, *I. chinensis*, *S. scandens* and *T. diversifolia* were prepared according to the methods described by Chang and Yeung (1988) with minor modification. Briefly, 100 g dried crude plants were decocted for 1 hour with 1000 ml of distilled water. The decoction was then filtered with gauze, concentrated under negative pressure and finally lyophilized to dry. The resulting lyophilized HW extracts were stocked at 4°C. They were dissolved in distilled water to desired concentration, and boiled for 5 minutes before use.

*Cells and Viruses*

The five leukemia cell lines used in anti-leukemic study were K562 (ATCC CCL 243), L1210 (ATCC CCL 219), P3HR1 (ATCC HTB 62), Raji (ATCC CCL 86) and U937 (ATCC CRL 1593). Human basal carcinoma cell line (BCC-1/KMC) (Chiang *et al.*, 1994), which is derived from facial skin, was used for antiviral assay. All cells were cultivated with RPMI 1640 (Gibco BRL) supplemented with 10% fetal calf serum (FCS) and 1% antibiotics (100 units/ml penicillin G sodium, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin sulfate), in a 37°C and 5% $CO_2$ incubator. Herpes simplex virus type-1 (KOS strain) and type-2 (196 strain) were prepared and quantitated on BCC-1/KMC cell.

*Titration of Virus*

BCC-1/KMC cell was seeded in 96-well plates at a density of $10^4$ cells/well, and then incubated in 5% $CO_2$ and 100% humidity at 37°C for 24 hours. After 24 hours, cell monolayers were infected with serially diluted virus stocks. The infected cells were incubated for another 72 hours until the cytopathic effect (CPE) was observed. The CPE was recorded, and the resulting $TCID_{50}$ was calculated as described by Reed and Muench (1938). Virus was stored at –80°C as small aliquots until use.

*Anti-leukemic Assay*

All leukemia cells were seeded into 96-well plates (Corning Co. USA) at concentration of $1 \times 10^4$ cells/well. Different concentrations of HW extract were then applied to culture wells with each concentration in triplicate. After incubation in 5% $CO_2$ and 100% humidity at 37°C for 72 hours, the viability of the cells was determined by cell proliferation XTT kit (Boehriger Mannhein GmbH) according to the manufacturer's protocol. The optical densities were determined with the EIA 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:

$$\text{Percentage of inhibition (\%)} = \frac{OD_T}{OD_C} \times 100\%$$

whereby $OD_T$ and $OD_C$ indicated the optical density of tested HW extract and solvent control, respectively. The concentration of HW extract that inhibited the growth of leukemia cells ($GI_{50}$) by 50% was calculated according to Chang *et al.* (2001).

*Antiviral Assay*

Anti-HSV activity of HW extract was evaluated with XTT-based colorimetric method (Weislow *et al.*, 1989). Briefly, BBC-1/KMC cells were seeded into 96-well plates at concentration of $1 \times 10^4$ cells/well. After 6 hours of incubation, the cells were infected by 25 $TCID_{50}$ HSV-1 or 20 $TCID_{50}$ HSV-2, respectively. The infected cells were further incubated for another 2 hours, and then serial concentrations of HW extract were added. After 72 hours of incubation, the medium was discarded, and cell proliferation XTT kit was applied. The trays were re-incubated for an additional 2 hours to allow the production of formazan. The optical densities were then measured with EIA reader (Multiskan EX, Labsystems) at a test wavelength of 450 nm and a reference wavelength of 690 nm. The antiviral activity of tested HW extracts was determined with the following formula (Pauwels *et al.*, 1988):

$$\text{Antiviral activity (\%)} = \frac{(OD_T)_{HSV} - (OD_C)_{HSV}}{(OD_C)_{mock} - (OD_C)_{HSV}} \times 100\%$$

whereby $(OD_T)_{HSV}$ is the optical density measured with a given concentration of the HW extract in HSV-infected cells; $(OD_C)_{HSV}$ is the optical density measured for the control untreated HSV-infected cells; $(OD_C)_{mock}$ is the optical density measured for control untreated mock-infected cells. The minimum concentration of HW extract required to inhibit 50% virus growth ($IC_{50}$) was calculated according to Cheng *et al.* (2002).

*Cytotoxic Effect*

The cytotoxic effect of tested HW extracts against BBC-1/KMC cells was evaluated with XTT-based colorimetric method. It was performed according to the procedures as described in "Antiviral Assay" section, except that no virus was inoculated. The concentration of tested HW extract that achieved 50% cytotoxic effect against BBC-1/KMC ($CC_{50}$) was calculated according to Cheng *et al.* (2002).

*Statistical Analysis*

Data were calculated as mean ± standard deviation for three separate experiments. Selectivity index (SI) of tested HW extract toward BBC-1/KMC was calculated as the ratio of $CC_{50}$ to $IC_{50}$. Student's unpaired *t*-test was used to calculate *p* value of difference of means between

control and sample. Difference between tested medicinal plant and *B. pilosa* with $p < 0.05$ was considered statistically significant.

## Results

### *Anti-leukemic Activity of Traditionally Used Medicinal Plants in Taiwan*

Table 1 shows the *in vitro* anti-leukemic activity (anti-K562, L1210, P3HR1, Raji and U937 cells) of HW extract of five traditionally used medicinal plants in Taiwan. HW extract of *B. pilosa* was used as a positive control.

Among the tested medicinal plants, *B. lacera* exhibited a broad spectrum of anti-leukemic activity. It possessed moderate to mild anti-L1210, Raji, P3HR1 and K562 activities, with $GI_{50}$ values of 175.5 ± 2.7, 210.4 ± 10.1, 216.5 ± 9.8 and 302.2 ± 6.8 μg/ml, respectively. The $GI_{50}$ value against U937 cell was > 500 μg/ml.

When tested against K562, L1210, P3HR1, Raji and U937 leukemia cells, the $GI_{50}$ values of *I. chinensis* were 84.8 ± 4.7, 177.0 ± 0.1, > 500, > 500 and 429.7 ± 15.2 μg/ml, whereas of *S. scandens* were > 500, 176.2 ± 3.8, 361.5 ± 16.9, > 500 and > 500 μg/ml, respectively. Our result showed that *I. chinensis* was more potent than *B. pilosa* to inhibit the growth of K562 cells ($p < 0.05$), and its $GI_{50}$ value was two-fold lower than that of *B. pilosa*. Also, only *I. chinensis* had $GI_{50}$ value less than 500 μg/ml against U937 leukemia cell. Its anti-U937 activity, however, was weak.

The anti-leukemic activity of the other two medicinal plants, *E. prostrata* and *T. diversifolia*, was weak. *E. prostrata* had a $GI_{50}$ value of 381.6 ± 3.6 μg/ml against L1210, and *T. diversifolia* had a $GI_{50}$ value of 356.7 ± 16.9 μg/ml against P3HR1. Both medicinal plants had $CC_{50}$ values > 500 μg/ml against the other four leukemia cells.

### *Antiviral Activity of Traditionally Used Medicinal Plants in Taiwan*

Table 2 shows the *in vitro* anti-HSV-1 and anti-HSV-2 activities of HW extract of *B. lacera, B. pilosa, E. prostrata, I. chinensis, S. scandens* and *T. diversifolia*. *B. pilosa* was tested as a positive control.

The $IC_{50}$ values of *B. lacera, E. prostrata, I. chinensis, S. scandens* and *T. diversifolia* against HSV-1 were 83.2 ± 1.5, 337.7 ± 8.4, 154.6 ± 4.8, > 500 and 94.4 ± 0.9 μg/ml, whereas against HSV-2 were 43.3 ± 25.1, > 500, 120.7 ± 6.3, 197.6 ± 3.5 and 34.8 ± 0.6 μg/ml, respectively. It was interesting to note that both *B. lacera* and *T. diversifolia* had $IC_{50}$ values lower than 100 μg/ml against HSV-1 and HSV-2.

The cytotoxic effect of tested medicinal plants against BCC-1/KMC cells was studied to ensure the anti-HSV activity was not a result of direct cytotoxic effect. Our result demonstrated that all tested medicinal plants had $CC_{50}$ values higher than 800 μg/ml (Table 2).

According to the resulting $IC_{50}$ and $CC_{50}$ values, SI values were calculated as ratio of $CC_{50}$ to $IC_{50}$ (Table 2). The SI values against HSV-1 and HSV-2 for *B. lacera* were > 12.0 and > 23.1, and for *T. diversifolia* were 9.9 and 27.1, respectively.

**Table 1. *In Vitro* Anti-leukemic Activity of Traditionally Used Medicinal Plants in Taiwan**

| | $GI_{50}$ (μg/ml)* | | | | |
|---|---|---|---|---|---|
| | **K562** | **L1210** | **P3HR1** | **Raji** | **U937** |
| *B. lacera* | 302.2 ± 6.8 | 175.5 ± 2.7 | 216.5 ± 9.8 | 210.4 ± 10.1 | > 500 |
| *B. pilosa*† | 171.5 ± 2.1 | 197.3 ± 2.1 | 196.5 ± 2.0 | 145.7 ± 2.2 | > 500 |
| *E. prostrata* | > 500 | 381.6 ± 3.6 | > 500 | > 500 | > 500 |
| *I. chinensis* | 84.8 ± 4.7‡ | 177.0 ± 0.1 | > 500 | > 500 | 429.7 ± 15.2 |
| *S. scandens* | > 500 | 176.2 ± 3.8 | 361.5 ± 16.9 | > 500 | > 500 |
| *T. diversifolia* | > 500 | > 500 | 356.7 ± 16.9 | > 500 | > 500 |

*Fifty percent growth inhibitory concentration ($GI_{50}$) was the concentration of HW extract that inhibited the growth of leukemia cells by 50%.

†*B. pilosa* was used as positive control. Each value represents the mean ± SD of three separate experiments.

‡$p < 0.05$ (compared with *B. pilosa*).

***Table 2. In Vitro Anti-herpes Simplex Virus Type-1 (HSV-1) and Type-2 (HSV-2) Activities, Cell Cytotoxic Effect and Selectivity Index of Traditionally Used Medicinal Plants in Taiwan***

| | | **HSV-1** | | **HSV-2** | |
|---|---|---|---|---|---|
| | $CC_{50}$ (μg/ml)* | $IC_{50}$ (μg/ml)† | SI‡ | $IC_{50}$ (μg/ml)† | SI‡ |
| *B. lacera* | > 1000 | 83.2 ± 1.5‖ | > 12.0 | 43.3 ± 25.1‖ | > 23.1 |
| *B. pilosa*§ | > 1000 | 655.4 ± 52.1 | > 1.5 | 960.0 ± 99.3 | > 1.0 |
| *E. prostrata* | 845.1 ± 45.1 | 337.7 ± 8.4‖ | 2.5 | > 500 | —¶ |
| *I. chinensis* | > 1000 | 154.6 ± 4.8‖ | > 6.5 | 120.7 ± 6.3‖ | > 8.3 |
| *S. scandens* | > 1000 | > 500 | —¶ | 197.6 ± 3.5‖ | > 5.1 |
| *T. diversifolia* | 942.8 ± 62.7 | 94.4 ± 0.9‖ | 9.9 | 34.8 ± 0.6‖ | 27.1 |

*Fifty percent cytotoxic concentration ($CC_{50}$) was the concentration of HW extract that showed 50% cytotoxicity against BCC-1/KMC cell.

†Fifty percent inhibitory concentration ($IC_{50}$) was the concentration of HW extract that inhibited 50% HSV infection in BCC-1/KMC cell.

‡Selectivity index (SI) = $CC_{50}/IC_{50}$.

§*B. pilosa* was used as positive control.

¶Not done. Each value represents the mean ± SD of three independent experiments.

‖$p < 0.05$ (compared with *B. pilosa*).

## Discussion

Cancer is the leading cause of death in the developing and developed countries. Every year, it claims over six million lives globally (Pezzuto, 1997). It was estimated that more than one million new cases of cancer would be diagnosed, and more than 0.5 million cancer-related deaths would occur (Miller *et al.*, 1992). On the other hand, the epidemic of HIV (Stoneburner *et al.*, 1994), the outbreak of an avian strain of influenza virus in Hong Kong (CDC, 1997) and Nipah virus in Malaysia and Singapore (CDC, 1999), and the eruption of SARS (Severe Acute Respiratory Syndromes) (CDC, 2003a and 2003b) have raised the problem of viral

infection. Although the FDA has approved many medicines for the management of cancer and viral infections, the side effects and the isolation of drug resistant viruses, however, have caused reduced efficacy or even failure of these medicines as treatments. Thus, the efforts of finding new drugs to treat cancer and viral infections are desired and the search is ongoing in many countries.

Traditionally used medicinal plants have been reported to exhibit a variety of biological activities, including antitumor and antiviral activities. The discovery of the efficacy of paclitaxel (Taxol), vincristine (Oncovin), vinorelbine (Navelbine), teniposide (Vumon), and various water-soluble analogues of camptothecin (such as Hycamtin) in cancer chemotherapy (Garattini and La Vecchia, 2001) has demonstrated that traditional medicinal plants play an important role in new drug development. Also, medicinal plants can serve as a potential resource in the development of new antiviral agents (Vlietinck and Vanden Berghe, 1991; Kaij-a-Kamb *et al.*, 1992).

In this study, HW extract of *B. lacera* was found to exhibit *in vitro* anti-leukemic and anti-HSV activities. *B. lacera* is traditionally employed as an astringent in hemorrhages, and as a deobstruent and stimulant. It is also applied for mumps, pneumonia, hepatitis, skin itch, bronchitis and inflammation of oral cavity (Chiu and Chang, 1992a). However, the anti-leukemic and anti-HSV activities of *B. lacera* have never before been reported.

Although *B. lacera* only exhibited moderate to mild anti-leukemic activity, this did not mean that it had little value for further investigation. The concentration of active compound(s) might be too low or the active component(s) might be lost during extraction or they might require *in vivo* metabolism to become active (Marsoni and Wittes, 1984; Double, 1992). In addition, *B. lacera* was considered to exhibit strong anti-HSV activity. Therefore further investigation on anti-leukemic activity and anti-HSV activities of *B. lacera* are encouraged.

Besides *B. lacera*, *T. diversifolia* was another noteworthy medicinal plant. *T. diversifolia* is a perennial herb widely distributed in the sunny grasslands of Taiwan. It is traditionally used as a remedy for hepatitis and hepatoma (Chiu and Chang, 1992b), and has been found in literature to exhibit a variety of biological activities, including cellular cytotoxic (Wu *et al.*, 2001; Gu *et al.*, 2002), anti-diabetic (Miura *et al.*, 2002), anti-HIV-1 (Cos *et al.*, 2002), anti-inflammatory (Rungeler *et al.*, 1998), anti-plasmodial (Goffin *et al.*, 2002), anti-ameobic, antibacterial and spasmolytic (Tona *et al.*, 1999 and 2000) activities. Recently, several pure compounds, categorized as sesquiterpene lactone, flavones and chromenes, have been isolated from *T. diversifolia* (Baruah *et al.*, 1979; Chowdury *et al.*, 1980; Schuster *et al.*, 1992; Bordoloi *et al.*, 1996; Kuo and Chen, 1997; Pereira *et al.*, 1997; Kuo and Chen, 1998). Among the purified compounds, some of them are found to inhibit the proliferation of human hepatocellular carcinoma (Hep G2) cell (Wu *et al.*, 2001) and human colon cancer (Col2) cell (Gu *et al.*, 2002) *in vitro*. However, our study demonstrated that HW extract of *T. diversifolia* showed weak anti-leukemic activity. These observations indicated that *T. diversifolia* exhibited a selective cytotoxic effect. Although HW extract of *T. diversifolia* was less effective at inhibiting the growth of leukemia cells, it was, however, active in suppressing the replication of HSV-1 and HSV-2. The extract of *T. diversifolia* also showed anti-HIV-1 activity (Cos *et al.*, 2002), an encouragement for further studies on antiviral properties of *T. diversifolia*.

In summary, *B. lacera* and *I. chinensis*, and *B. lacera* and *T. diversifolia* were concluded to inhibit leukemia cell growth and to suppress HSV infection at different magnitudes of potency, individually. Further fractionation and purification of the active components, and clarification on the mode of action for *B. lacera*, *I. chinensis* and *T. diversifolia* therefore merit investigation.

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