



Comparative study of *Tilia x viridis* extract and isolated compounds on the proliferation of two lymphoma cell lines.

Patricia Micucci, Paola Di Leo Lira, Gabriela Zettler, Sebastian Turner, Graciela Ferraro, Roberto Davicino, Claudia Anesini*.

IQUIMEFA-UBA-CONICET and Pharmacognosy Unit, Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

ABSTRACT

Several lymphoma T cell lines differ in membrane superficial markers and do not respond to the same drugs and compounds. *Tilia x viridis* (Bayer) Simonk nothosubspecie moltkei (Dippel) Xifreda is a plant widely distributed in Argentina. The aim of this work was to study comparatively the effects of dichloromethane extract and isolated compounds on BW 5147 and on another T lymphoma cell line EL-4, evaluating principally proliferation and viability. On BW 5147 cells, dichloromethane extract exerted antiproliferative action, presented cytostatic effect at low concentrations and cytotoxic action at high concentrations. Monoterpenes exerted antiproliferative action being the most potent limonene; low concentrations of them were cytostatic and high concentrations were cytotoxic. On EL-4 lymphoma, dichloromethane extract and monoterpenes exerted proliferative action, and none of them affected cell viability. It is extremely important to note that depending on tumor cell type the same extract or compounds can elicit different actions.

Keywords: : *Tilia x viridis*, dichloromethane extract, monoterpenes, BW 5147, EL-4.

Corresponding author: Anesini C. IQUIMEFA-UBA-CONICET and Pharmacognosy Unit, Faculty of Pharmacy and Biochemistry, Junín 956, 2nd floor, CP: 1113, University of Buenos Aires. canesini@yahoo.com.ar, Tel: 54-011-49648247

Received: February 22, 2010. Accepted: March 10, 2010



Introduction

Several lymphoma T cell lines differ in membrane superficial markers and do not respond to the same drugs and compounds (Ralph, 1973). On the other hand, some plants are used in popular medicine for the treatment of several diseases. In this sense, *Tilia* species have been used for many years in Europe to treat anxiety and as a psychological depressor (Font Quer, 1976) but also, the extracts have been used for the treatment of colds, bronchitis, fever, inflammations and influenza infections. In this regards, the tea prepared with *Tilia* flowers is listed in German Pharmacopeia (Thomson, 1987). *Tilia x viridis* (Bayer) Simonk nothosubspecie moltkei (Dippel) Xifreda is a plant widely distributed in Argentina. In a previous study, it was shown that a dichloromethane extract of *Tilia x viridis* presented antiproliferative action on a lymphoma cell line (BW 5147). Moreover, one fraction rich in coumarins and other rich in monoterpenes were obtained from dichloromethane extract. The fraction most active against tumor cells was the fraction containing monoterpenes including limonene (Manuele *et al*, 2008). The aim of this work was to study comparatively the effects of dichloromethane extract and isolated compounds (monoterpenes such as limonene, α pinene and β pinene) on BW 5147 and on another T lymphoma cell line EL-4, evaluating principally proliferation and viability.

Experimental

Tilia x viridis (Bayer) Simonk nothosubspecie moltkei (Dippel) Xifreda flowers were collected in the province of Buenos Aires in January and authenticated by Dr Gustavo Giberti (IQUIMEFA-CONICET). A voucher specimen is deposited at Museum of Pharmacobotanic, Faculty of Pharmacy and Biochemistry, University of Buenos Aires. To prepare the dichloromethane extract (DM), dried flowers (9g) were macerated twice overnight with 200 ml of dichloromethane, filtered, evaporated and the residue used for the experimental studies. Moreover, the monoterpenes were quantified by gas chromatography (Manuele *et al* 2008).

Monoterpenes: Limonene, α -pinene and β -pinene were purchased in Sigma-Aldrich company (San Diego, USA)

Cell culture conditions

The lymphoma T cell lines (BW 5147 and EL-4) were obtained from Institute fur Virologie und

Immunobiologie der Universitat Wurzburg, Germany. Cells were cultured at optimal concentrations of 3×10^5 cells/ml in RPMI 1640 medium (Sigma, San Diego, USA) supplemented with 10 % fetal calf serum, 2 mM glutamine and antibiotics (Sigma, San Diego, USA) (Anesini *et al*, 1996).

Proliferation assays

The effect on proliferation of different concentrations (1, 10, 100, 1000 and 10000 $\mu\text{g/ml}$) of DM and monoterpenes was evaluated. Cell proliferation was assayed by the uptake of tritiated thymidine (^3H TdR). Cells were cultured during 24 h and then pulsed with ^3H TdR (20 Ci/mmol) for the last 6 h as previously described (Anesini *et al*, 1996). Results were expressed as dpm and as percentage of proliferation inhibition respect to control.

Viability assay

Cell viability of treated and untreated cells were determined by the reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma, San Diego, USA). Briefly, tumor lymphocytes (3×10^5 cells/ml) were incubated alone or in presence of different concentrations of DM or monoterpenes during 24 h. After incubation, 100 μl of RPMI 1640 containing 10 μl of MTT (5 mg/ml) (Sigma, CA, USA) were added. The purple formazan formed was solubilized after 4 h of incubation by the addition of acidic isopropanol. The absorbance was measured using a microplate reader (Microplate Reader Benchmark. Bio-Rad) at 570 nm. Untreated cells were used as control of viability (100%) and results were expressed as % of viability relative to control (Davicino *et al*, 2008).

Statistic analysis

Data are the average of duplicate samples performed by triplicate. The data were recorded as mean value \pm standard error of mean. One way analysis of variance was performed by ANOVA followed by comparisons with Dunnett test. A $p \leq 0.05$ was considered statistically significant.

Results and discussion

On BW 5147 cells, DM extract exerted antiproliferative action (EC_{50} $\mu\text{g/ml}$: 4.8 ± 2), presented cytosatic effect at low concentrations and cytotoxic at high concentrations. Monoterpenes exerted antiproliferative action



being the most potent limonene (EC_{50} $\mu\text{g/ml}$: 35 ± 2 , β pinene: 114.8 ± 8 , α pinene: 1100 ± 10), low concentrations of them were cytostatic and high concentrations were cytotoxic (Figure 1 and Table 1).

On EL-4 lymphoma, DM and monoterpenes exerted proliferative action, and none of them affected cell viability. The maximum stimulatory concentration for the extract was $100 \mu\text{g/ml}$ (Figure 2). On the other hand, monoterpenes were found in low concentrations in DM (Figure 3).

On BW 5147, monoterpenes could contribute to the antiproliferative activity of high concentrations of DM, at low concentrations of DM other compounds more active should exert the effect or monoterpenes could have a synergistic action. On EL-4 cells, the stimulatory action on cell proliferation could be related to limonene, to the synergistic effect of all monoterpenes or to the presence of other kind of compound. It is extremely important to note that depending on tumor cell type the same extract or compounds can elicit different actions.

Acknowledgement

This work was supported by funds provided by PIP CONICET 5232. Roberto Davicino is a postdoctoral fellow of CONICET.

Note: Part of this study was presented at the 'II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

References

- Anesini C, Genaro A, Cremaschi G, Sterin Borda L, Cazaux C, Borda E. Immunomodulatory activity of *Larrea divaricata*. (1996). *Fitoterapia* **67**: 329-333.
- Davicino R, Manuele MG, Ferraro G, Micalizzi B, Anesini C. (2009). Modulatory effect of hydrogen peroxide on tumoral lymphocytes proliferation. *Immunopharmacol Immunotoxicol* **31**: 130-139.
- Font Quer R. (1976), Plantas medicinales. El dioscórides renovado; Labor; Barcelona, España.
- Manuele MG, Ferraro G, Anesini C. (2008). Effect of *Tilia x viridis* flower extract on the proliferation of a lymphoma cell line and on normal murine lymphocytes: contribution of monoterpenes, especially limonene. *Phytother Res* **22**: 1520-1526.
- Ralph P. (1973). Retention of Lymphocyte Characteristics by Myelomas and {theta}+ Lymphomas: Sensitivity to Cortisol and Phytohemagglutinin. *J Immunol* **110**: 1470-1475.
- Thomson A. (1987), *Healing plants (a modern herbal)*; Mc Graw Hill Book Company; London, England.

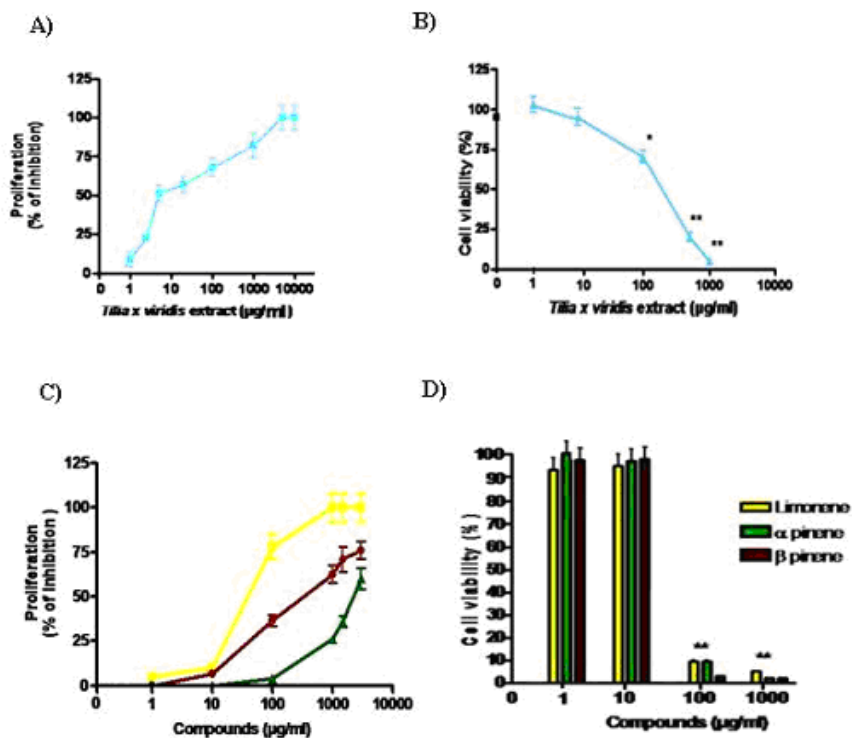


Figure 1. Effect of DM and monoterpenes on proliferation (A, C) and viability (B, D) of BW5147 cell line. Results represent the Mean \pm SEM of three experiments made by triplicate. * $P < 0.05$, ** $p < 0.01$ significantly differences between basal and treated groups

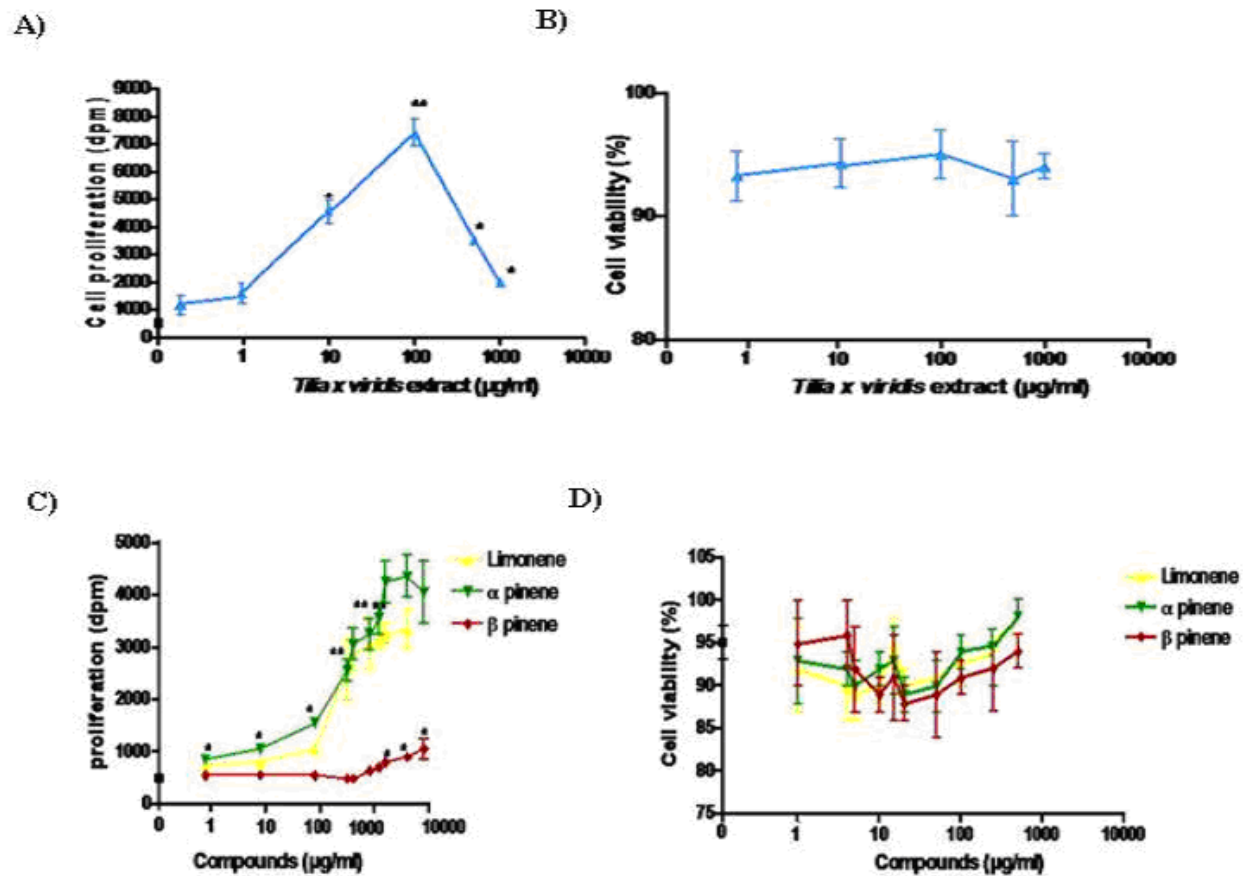


Figure 2. Effect of DM and monoterpenes on proliferation (A, C) and viability (B, D) of EL-4 cell line. Results represent the Mean \pm SEM of three experiments made by triplicate. * $P < 0.05$, ** $p < 0.01$ significantly differences between basal and treated groups.

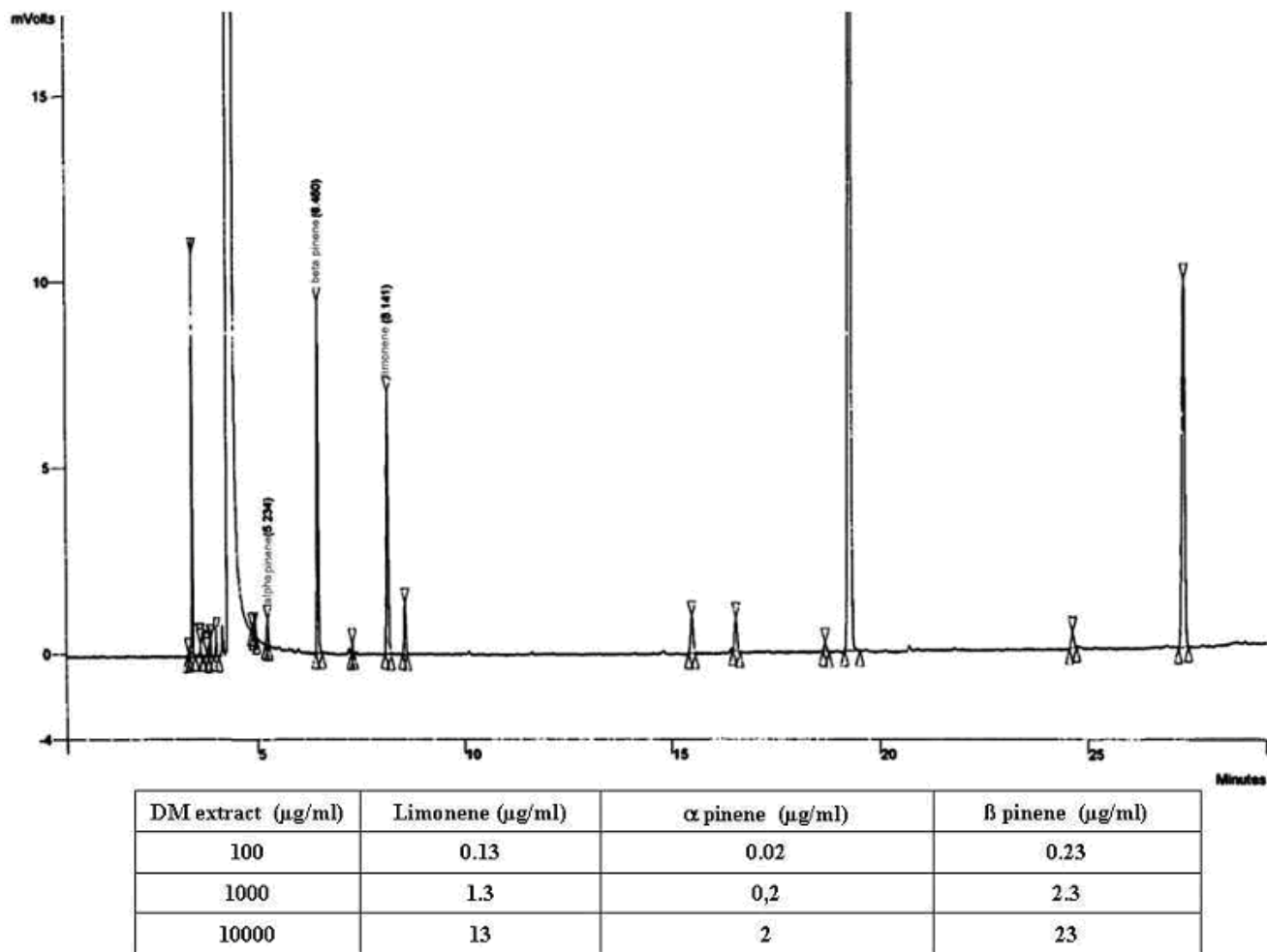


Figure 3. Chromatogram of DM of *Tilia x viridis* extract. The chromatogram was obtained by gas chromatography

**Table 1.** EC₅₀ of *Tilia* extract and monoterpenes on the antiproliferative action in BW 5147 lymphoma cells.

Additions	EC ₅₀ (µg/ml)
DM extract	4.8 ± 2
α-pinene	1100 ± 10
β-pinene	114.8 ± 8
Limonene	35 ± 2