



Minthostachys verticillata: toxicity of its essential oil and major constituents to *Artemia salina* and cell lines

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INTRODUCTION

A viral infection treatment requires the use of drugs, which selectively affect the pathogen agent without altering the host-cell. At present these substances are scarcely available, thus the search of plant compounds as a possible phytopharmaceutical source is being of great interest (Jassim and Naji, 2003). Previous studies have demonstrated that fractions of different plant species, e.g., *Minthostachys verticillata* (Griseb) Epling show antiviral *in vitro* activity (Zanon *et al.*, 1999). These results encouraged to deepen the cytotoxic action study of the essential oil (EO) of *M. verticillata* (Labiatae) and its major constituents used individually or in mixtures on Vero and HEP-2 cell cultures, and in parallel to evaluate the cytotoxic potential of the same samples on *Artemia salina* crustacean.

METHODOLOGY

In this study the EO and its major constituents, pulegone (P), menthone (M) and limonene (L), were used. The analysis of cytotoxicity on *A. salina* larvae was carried out according to the methodology described by Franssen *et al.* (1997). For counting total larvae and those which died by treatment, the technique developed by Visconti *et al.* (1992) was followed. The 50% Lethal Dose (LD₅₀) was calculated according to the Reed-Muench (1938) method. The obtained values allowed to estimate the concentrations to be tested on cell cultures. Treatment of the latter for 72 h allowed to calculate the Maximum Non-Cytotoxic Concentration (MNCC) and the 50 % Cytotoxic Concentration (CC_{50%}).

The determination of MNCC in both cell lines was carried out by observation of the treated monolayers *versus* controls under optic microscope.

The CC_{50%} determination was developed on HEP-2 cell line, and the values were calculated by simple lineal regression analysis of the dose-response graphs obtained by viable counting by the trypan blue exclusion method.

RESULTS AND DISCUSSION

The LD₅₀ values (Table 1) indicate that limonene showed to be the less toxic compound, while pulegone exhibited this action at a lower concentration, thus revealing its higher toxicity (Fig. 1).

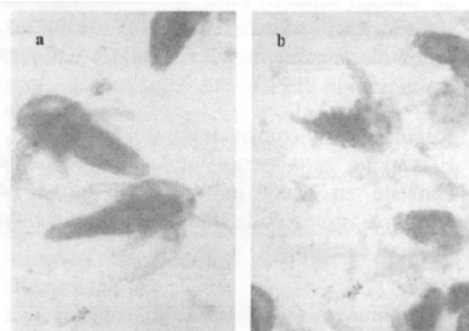


Figure 1. Larvae of *A. salina*: **a)** control, **b)** treated with pulegone at toxic concentration.

Similar studies carried out with *Thymus fallax* (Labiatae) derivatives reported LD₅₀ of nearly 0.5-0.9 mg/ml, which are values that reveal a major degree of toxicity on this crustacean (Sökmen, 2001).

The toxicity analysis as function of the MNCC values on cell cultures revealed that all tested samples, except for menthone, were more toxic on Vero cells than on HEP-2 cells (Table 2). On the other hand, the three major constituents in pure form induced higher cytotoxicity than the EO on both cell lines, inferring that the smallest cytotoxicity exerted by the EO could be due to an antagonistic action among the major constituents.

The other constituents of this oleous mixture -not analysed in the present work- could show the same behaviour.

**Table 1.** LD₅₀ of the EO of *M. verticillata*, its major constituents and combinations on *Artemia salina*.

Plant Compound	LD ₅₀ (mg/ml)
EO	2.10
Pulegone (P)	0.30
Menthone (M)	1.12
Limonene (L)	5.25
P-M	0.47 - 0.12
P-L	ND
M-L	5.01 - 0.54

ND: Not Determined

Table 2. Toxicity of the EO of *M. verticillata* and its major constituents on cell lines at 72 h post-treatment.

Sample	MNCC (µg/ml)	
	Vero	HEp-2
EO	811	1,013
Pulegone	60	132
Menthone	400	370
Limonene	250	450

Although the MNCC is a valid toxicity marker, the CC₅₀ value is more precise than MNCC because of being a more objective method.

According to results shown in Table 3, limonene and the mixture menthone-limonene were the less toxic of all pure compounds and of the analysed combinations, respectively, tested by the methods mentioned above.

Table 3. Toxicity values of the constituents of the EO of *M. verticillata*, tested in singular and combined forms on HEp-2 cells.

Compound	MNCC (µg/ml)	CC ₅₀ (µg/ml)
Pulegone (P)	132	131
Menthone (M)	370	372
Limonene (L)	450	1,280
P-M	100-24.4	127-31
P-L	96-2.68	97-2.7
M-L	220-24	420-45
P-M-L	50-12.2-1.	62-15-1.6

CONCLUSIONS

On the basis of these results, it can be drawn the conclusion that the LD₅₀ values on *A. salina* allowed to approximate the sample concentration ranges for tests on cell cultures, which showed that the essential oil of *M. verticillata* and limonene were those of less toxicity. This low toxicity encourages to investigate the antiviral activity, and to confirm by the therapeutic index the possibility of being used as phytopharmaceuticals, taking into account the availability of this shrub in our mountain area.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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