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Minthostachys verticillata essentials oil and its major components: antiherpetic selective action in HEp-2 cells.

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ABSTRACT

The medicinal herb *Minthostachys verticillata* was studied in various fields of natural medicine. Numerous studies were made to evaluate its antimicrobial properties. This work emphasizes in the analysis of the antiviral capacity of its Essential Oil (EO). The aim of this study was to determine the Therapeutic Index (TI) of the EO and its main chemical components. *Suid herpesvirus type 1* or *Pseudorabies virus* (Prv) was proliferated in HEp-2 cells in presence of non-toxic concentrations of the oily samples. Antiviral action was quantified by reducing the number of plaques of lysis. Previously, the cytotoxicity of the samples was evaluated and 50% Cytotoxic Concentration values (CC_{50}) were determined. The TI was calculated by relating the cytotoxic concentrations of each sample *vs.* those concentrations active against the virus. The results showed that the major components of the EO were pulegone, menthone and limonene, with CC_{50} of 352, 835 and 1155 µg/mL respectively *vs.* the CC_{50} of EO: 613 µg/mL. The antiviral action was exerted by pulegone and the EO at concentrations 20 to 30 times lowers than the toxic concentrations. These IT values show the possible applicability of these compounds because they exert selective antiviral action.
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Keywords: *Minthostachys verticillata*, essential oil, *Herpes suis* virus *type 1*, antiviral action, therapeutic index.

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Introduction

Considering that the 80% of the world's population depends on medicinal plants for it primary health care, the search of new substances of plant origin that may possess antiviral activity has been intensified in recent years (Núñez and Cantero, 2000).

From the wide and varied range of medicinal and aromatic plants with potential pharmacological activity that Córdoba province (Argentina) offer; the *Minthostachys verticillata* study is very important. It belongs to Lamiaceae family and is popularly known as peperina. It has antibacterial, antifungal and antiviral properties (De Feo *et al*., 1998; Zanon *et al*., 1999; Primo *et al*., 2001).

Because of viral agents are obligate intracellular parasites, it is very difficult to find compounds with selective activity against viruses without affecting the host cells. The Therapeutic Index (TI) is a parameter that relates the toxic capacity of the compound under study and their inhibitory activity of viral replication, allowing inferring its potential clinical application.

The aim of this study was to determine the antiviral action of *M. verticillata* Essential Oil (EO) and its main components on *Suid herpesvirus type 1* replication cycle employing HEp-2 cell monolayers and also to determine the TI.

Experimental

1. Plant samples

Green leaves and thin stems of *Minthostachys verticillata* (Griseb.) Epling (Labiatae) were collected, during morning hours, from the city Santa Rosa in Córdoba province, Argentina, in April 2007. The plant was identified by Dr. Margarita Grosso, professor in the Area of Botany of the Universidad Nacional de Río Cuarto, and a voucher specimen was stored in the RCV (Río Cuarto Vasculares) herbarium as file #1955. The morphological characterization of the plant was executed macro-and microscopically to confirm the identity of these specimens. The aerial parts of the plant were made up of the leaves and parts of the stem. The oil was isolated from the aerial parts.

2. Essential oil isolation

To obtain the essential oil (EO), 60 g of ground plant material were hydro-distilled for 3 h using a Clevenger-type apparatus, yielding 4.8% of the oil. The oil was stored in the dark at -20 °C until use. Quantification of components present in the oil sample was made by gas chromatography. Previous studies by this method showed that pulegone, menthone and limonene were the main compounds. The ratio found was: 62.97%, 16.40% and 1.87% respectively (Cariddi *et al*., 2009). In order to perform the cytotoxic and antiviral *in vitro* assays, various concentrations of oily samples were obtained. First they were emulsified in Dimethylsulfoxide (DMSO) and then diluted in Maintenance Medium (MM) Eagle-Earle supplemented with 2% fetal bovine serum (FBS), glutamine and antibiotics.

3. Cytotoxicity assay

Cellular viability was measured with the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma-Aldrich) (MTT) method (Seth *et al.*, 2004). Confluent cultures of HEp-2 cells grown with Eagle-Earle medium supplemented with 8% FBS, glutamine and antibiotics in 96-well plates were exposed during 48 h at 37°C to different concentrations of the oily samples. Were employed three wells for each dilution; untreated cells were used as control. The Optical Density (OD) was measured at 560 nm using a microplate ELISA reader (Labsystems Multiskan MS, Finland).

The Survival Fraction % (SF%) in the treated cultures was calculated from the DO, in relation to cultures controls, that represent the 100% viability:

$$SF\ \% = \frac{\text{D.O. treated cells}}{\text{D.O. control cells}} \times 100$$

4. Antiviral activity assays

To determine the 50% Effective Concentrations (EC_{50}), confluent monolayers of HEp-2 cells developed in 24-well culture plates were infected with 200 µL of ten fold dilutions of *Suid herpesvirus type 1, RC/79* strain. After 90 min incubation at 37°C the remaining viral suspension was discarded and 1 mL of Plate Medium (PM): MM + 0.75% of methyl cellulose, containing the compound under study at non-cytotoxic concentrations were added. The assays included the followings controls: **Cellular Control,** cultures with PM only; **Viral Infectivity Control**, infected cultures covered with PM only; and **Cytotoxicity Control,** cultures covered with PM

Molecular Medicinal Chemistry
IDECEFYN
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containing the oily sample at the concentration tested (Garcia *et al,* 2000, Gong *et al.,* 2004).

After 72 h incubation at 37 °C, viral titters were calculated by the method of Dulbecco R. (1962). The evaluation of the inhibitory action exerted by the oily samples was performed by the plaque reduction assay (Chattopadhyay *et al.,* 2009). The degree of antiviral activity of each sample was determined by relating the viral titers obtained in treated cultures *vs*. viral titers obtained from controls of viral infectivity.

% Viral inhibition = 100 - A / B x 100

Where:
A = Title viral expressed in PFU/mL obtained in treated cultures.
B = Title viral expressed in PFU/mL obtained in untreated cultures.

5. Statistical analysis

The experiments of cytotoxic and antiviral activity were performed in triplicate. The CC_{50} and EC_{50} values were calculated using a nonlinear regression model based on Boltzmann sigmoideal curve by the software *GraphPadPrism 5.0*.

6. Determination of the TI of M. verticillata EO and its main components

The TI for the different oily samples was calculated according describes (Li *et al.,* 2002) using the following formula:

$TI = CC_{50} / EC_{50}$

Results and discussion

1. Analysis of the cytotoxic activity

Cytotoxicity values are summarized in Table 1. Pulegone was the most toxic compound due to the lower concentration (352 µg/mL) affected 50% HEp – 2 cells viability. The CC_{50} values obtained were of 613 µg/mL for the EO, 835 µg/mL for menthone and 1155 µg/mL for limonene, being the last one the less toxic. This order coincides with previously reported results (Sutil *et al.,* 2006).

2. Antiviral activity assays

In this study the results indicated that only the EO of *M. verticillata* and pulegone inhibited the replication of *Suid herpesvirus type 1* in more than 50%. Both samples exerted an inhibition over 90% at concentrations of 100 µg/mL. The EC_{50} of EO was 20.25 µg/mL and 20 µg/mL for pulegone. The other compounds showed a slight antiviral activity and limonene was the less active.

3. Determination of therapeutic index

The TI values for all samples were calculated. They were 30.3 for the EO and 17.6 for pulegone. Data revealed that only these samples could be used as therapeutic agents due to these selective actions.

Conclusions

The MTT technique was appropriate to determine the cytotoxicity values for the EO of *M. verticillata* and its main components. Menthone and limonene exerted little antiviral action and it was not selective. The EO of *M. verticillata* and pulegone showed similar EC_{50} values (≥20). The EO of *M. verticillata* and its main compound, pulegone, confirm selective antiviral action.

Data reveal the therapeutic potential and the ethnobotanical importance of the *Minthostachys verticillata* specie.

Table 1: *M. verticillata* EO and its main components, Therapeutic Index

SAMPLE	MTT CC_{50}[a] µg/mL	EC_{50}[b] µg/mL	TI[c]
EO[d] *M. verticillata*	613	20.25	30.3
Pulegone	352	20	17.6
Menthone	835	> 835	<1
Limonene	1155	> 1155	<1

CC_{50}[a]: Cytotoxic concentration 50%,
EC_{50}[b]: Effective concentration 50%,
TI[c]: Therapeutic Index,
EO[d]: Essential oil

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References

-Cariddi LN, Moser M, Andrada M, Demo M, Zygadlo J, Sabini L, Maldonado AM, (2009). The

Molecular Medicinal Chemistry
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effect of *Minthostachys verticillata* essential oil on the immune response of patients allergic to dust mites. *BLACPMA* **8(3):**224–233.

-Chattopadhyay D, Chawla-Sarkar M, Chatterjee T, Sharma Dey R, Bag P, Chakraborti S, Khan MTH, (2009). Recent advancements for the evaluation of anti-viral activities of natural products. *New Biotechnol* **25(5):**347-368.

-De Feo V, Ricciardi A, Biscardi D, Senatore F, (1998). Chemical composition and Antimicrobial screening of the Essential Oil of *Minthostachys verticillata* (Griseb.) Epl. (Lamiaceae). *J Essent Oil Res* **10**:61-65.

-Dulbecco R, (1962). Production of plaques in monolayer tissue culture by single particles of an animal virus. *Proc Natl Acad Sci USA*. **38**:747-752.

-García CC, Candurra NA, Damonte EB, (2000). Antiviral and virucidal activities against arenaviruses of Zinc-finger active compounds. *Antiviral Chem Chemother* **11**:231-238.

-Gong Y, Raj K, Luscombe C, Gadawski Y, Tam T, Chu J, Gibson D, Cortson R, Sacks S, (2004). The synergistic effects of betulin with acyclovir against Herpes simplex virus. *Antiviral Res* **64:**127-130.

-Li Y, Ma S, Yang Y, Ye S, But P, (2002). Antiviral activities of flavonoids and organic acid from Trollius chinensis Bunge. *J Ethnopharmacol* **79**:365-368.

-Núñez C, Cantero J, (2000). Las plantas medicinales del sur de la provincia de Córdoba. Edit. Universidad Nacional de Río Cuarto, Argentina.

-Primo V, Rovera M, Zanon S, Oliva M, Demo M, Daghero J, Sabini L, (2001). Determination of the antibacterial and antiviral activity of the essential oil from *Minthostachys verticillata* (Griseb.) Epling. *Rev Argent Microbiol* **33**:113-117.

-Seth R, Yang S, Choi S, Sabean M, Roberts E, (2004). In vitro assessment of copper-induced toxicity in the human hepatoma line Hep G2. *Toxicol in Vitro* **18**: 501-509.

-Sutil S, Astesano A, Vogt V, Torres C, Zanon S, Sabini L, (2006). *Minthostachys verticillata*: Toxicity of its essential oil and major contituentes to *Artemia salina* and cell lines. *IDECEFYN* **10(1):** 41-42.

-Zanon S, Ceriatti FS, Rovera M, Sabini L, Ramos B, (1999). Search for antiviral activity of certain medicinal plants from Córdoba, Argentina. *Rev Latinoam Microbiol* **41:**59-62.