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Reduction of *in vitro* viral replication of caprine alphaherpesvirus 1 (CPHV-1) through natural antiviral treatment with plant extracts

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Abstract
Caprine herpesvirus 1 (CpHV-1) is distributed worldwide, causing significant economic losses. The application of acyclovir interferes with viral replication to varying degrees of efficacy, but it does not prevent the establishment of latency. Due to the costs and side effects associated with synthetic drugs, the search for new chemotherapeutic agents is essential for the treatment and control of viral diseases. The objective of this study is to evaluate and compare the *in vitro* antiviral activity of three native plants from Argentina as a therapeutic antiviral treatment against CpHV-1. *Larrea divaricata, Minthostachys verticillata* and *Parastrephia quadrangularis* were used. Four parameters were analyzed: Cytotoxic Concentration 50 (CC50), Inhibitory Concentration 50 (IC50), quantification by plaque assay and viral titration. The *L. divaricata* extract showed the lowest cytotoxicity (2.4 mg ml^{-1}). The best selectivity index (SI) was obtained with the *M. verticillata* extract (10). *L. divaricata* and *P. quadrangularis* extracts demonstrated the greatest antiviral effects, with reductions of 90% and 75%, respectively, compared to untreated controls, where the greatest reduction in plaque size was observed. The highest inhibition of viral replication observed in the viral titration quantification occurred when monolayers were treated with *L. divaricata*, resulting in at least a 35% reduction. The combination of assay with *L. divaricata* and *M. verticillata* showed the greatest reduction in viral titers. The overall results provide evidence that *L. divaricata* and *M. verticillata* could serve as potential sources for new anti-CpHV-1 drugs.

Key words: herpesvius, treatment, *L. divaricata*, *M. verticillata*, *P. quadrangularis.*

Reducción de la replicación viral *in vitro* de alfaherpesvirus caprino 1 (CpHV-1) por tratamiento antiviral natural de extractos de plantas

Resumen. El herpesvirus caprino 1 (CpHV-1) se distribuye mundialmente siendo responsable de pérdidas económicas. La aplicación de aciclovir interfiere en la replicación viral con distintos grados de eficacia, pero no impide el establecimiento de latencia. Debido a los costos y efectos secundarios que producen los fármacos antihipertensivos, la búsqueda de nuevos agentes quimioterapéuticos es fundamental para el tratamiento y control de las enfermedades virales. El objetivo del trabajo es evaluar y comparar la actividad antiviral in vitro de tres plantas nativas de Argentina como tratamiento antiviral terapéutico contra CpHV-1. Se utilizaron *Larrea divaricata*, *Minthostachys verticillata* y *Parastrephia quadrangularis*. Se analizaron cuatro parámetros: Concentración Citotóxica 50 (CC50), Concentración Inhibitoria 50 (CI50), cuantificación por ensayo de placa y titulación viral. El extracto de *L. divaricata* mostró la menor citotoxicidad (2,4 mg ml^{-1}). El mejor SI (índice de selectividad) se obtuvo en el extracto de *M. verticillata* (10). Los extractos de *L. divaricata* y *P. quadrangularis* son los compuestos que mostraron el mayor efecto antiviral (90% y 75%, respectivamente), en comparación con los controles no tratados, en los que se observó la mayor reducción

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en el tamaño de la placa. La mayor inhibición de la replicación viral se observó cuando se evaluó el título viral cuando las monocapas fueron tratadas con *L. divaricata* (al menos disminución del 35%). Con el ensayo de combinación de *L. divaricata* y *M. verticillata* se observó la mayor reducción de los títulos virales. Los resultados generales proporcionan evidencia de que *L. divaricata* y *M. verticillata* podrían ser fuentes potenciales de nuevos fármacos anti-CpHV-1.

Palabras clave: herpesvius, treatment, *L. divaricata*, *M. verticillata*, *P. quadrangularis.*

INTRODUCTION

Caprine herpesvirus 1 (CpHV-1) is a virus belonging to the Herpesvirales order, Herpesviridae family, Alphaherpesvirinae sub-family, and Simplexvirus genus (Davison 2010). CpHV-1 is correlated with two different clinical entities in goats: a lethal systemic disease in newborn goats and a genital disease leading to balanoposthitis (Tarigan et al. 1987), vulvovaginitis (Grewal and Wells 1986), abortion (Keuser et al. 2002), weight loss, and reduced milk yield (Candanosa et al. 2016). The genital tropism of CpHV-1 was confirmed by detecting viral DNA in the sacral ganglia of latently infected goats (Tempesta et al. 1999). Interestingly, CpHV-1 shares several biological features with human herpes simplex virus 2 (HSV-2), such as tropism for the vaginal epithelium, the type of genital lesions, and the establishment of latency in the sacral ganglia (Whitley et al. 2001, Tempesta et al. 1999, Tempesta et al. 2000), serving as a model for the evaluation of antiviral compounds for human genital viral infections. It may contribute to significant economic losses at the farm level worldwide (Candanosa et al. 2016, Camero et al. 2019) due to its impact on female fertility and its ability to induce abortions during the final third of pregnancy. However, there are no estimates of the economic losses caused by this infection in Argentina. Currently, there is no specific treatment for goats, and although there are reports on the development of various classical inactivated vaccines, none are currently licensed (Camero et al. 2007). Although it has been reported that the application of acyclovir and cidofovir (Tempesta et al. 2007, Tempesta et al. 2008) interferes with viral replication to varying degrees of efficacy (reduction of lesions or excretion) in both *in vivo* and *in vitro* tests, it does not prevent the establishment of latency in goats (Camero et al. 2010). Recently, a greater inhibitory effect on CpHV-1 replication was observed *in vitro* when acyclovir was used in combination with mizoribine (Camero et al. 2017).

Conventional drugs are associated with numerous side effects, and the active mutation of viruses contributes to the development of drug resistance. In addition, due to the costs and side effects associated with acyclovir and cidofovir in both humans and animals, the search for new chemotherapeutic agents for the treatment and control of viral diseases is essential (Candanosa et al. 2016, Camero et al. 2019).

The great diversity of plants that grow in Argentina, due to its wide geographical extension and diversity of bioclimates, offers interesting possibilities to find antiviral compounds of natural origin (Visintini et al. 2013), which is why species can be selected for their ethnobotanical value and their antimicrobial, antifungal, anti-inflammatory, antioxidant, antiviral, and acaricidal properties. Throughout history, people have sought drugs in nature to treat different symptoms and diseases. In ancient times, medicinal plants were used instinctively, as there was not enough information about the reasons for the illness or how plants could be used for healing purposes. For Argentina, the work carried out with extracts from native plants of different plant species on the *in vitro* inhibition of viruses with significance in animal health stands out (Martinez et al. 2021, 2022). *Larrea divaricata* is a shrub that grows in South America and is widely distributed in Argentina. This plant is used in folk medicine for its anti-inflammatory, antitumor, immunomodulatory, antiviral and antimicrobial properties (Konigheim et al. 2006, Stege et al. 2006, Peralta et al. 2022). This is primarily attributed to the presence of nordihydroguaiaretic acid (NDGA), a metabolite found exclusively in the genus *Larrea* (Lü et al. 2010, Martinez et al. 2022). *Minthostachys verticillata,* which grows in northeastern Argentina and Paraguay, has a history of antiviral, antibacterial and anti-inflammatory activity (Vogt et al. 2010, Sim et al. 2019, Rodríguez Basso et al. 2021). *Parastrephia quadrangularis* (Meyen) (Asteraceae) is a native plant of the Puna grassland, a phytogeographic region of northern Argentina, at altitudes greater than 3200 m a.s.l. The genus *Parastrephia* has undergone various bioactivity evaluations, showing that the species has antimicrobial, antinflammatory, antioxidant and acaricide properties (Ayma et al. 1995, Zampini and Sayago 2008, Zampini et al. 2009, D'Almeida et al. 2012, Echiburu-Chau et al. 2017) as well as antifungal activity (Di Ciaccio et al. 2018). These plants were tested against human pathogens but have not yet been tested against veterinary pathogens. Therefore, the objective of this study was to evaluate the *in vitro* effectiveness of native plant extracts as a possible preventive and therapeutic antiviral treatment of CpHV-1.

MATERIALS AND METHODS

Plant material. *P. quadrangularis* extracts (Ardiles et al. 2018, Di Ciaccio et al. 2018) were provided by the Institute of Pathobiology, National Institute of Agricultural Technology (INTA). The ethanolic extracts of *L. divaricata* (Konigheim et al. 2006) and *M. verticillata* were provided by the National University of Córdoba, Faculty of Medical Sciences, Institute of Virology "Dr. J.M. Vanella".

Viruses and cells. Monolayers of MDBK (Madin-Darby Bovine Kidney) cells were used. They were grown in a humid atmosphere at 37 °C with 5% CO_2 using Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% gentamicin.

The assays were performed with virus BA.1 strain (10^7 DICT 50% ml^{-1}) of CpHV-1 (isolated from latently infected goats). The virus stock was obtained and titrated according to the methods described by Tempesta et al. (2001).

***In vitro* tests**

a) CC50, IC50 and SI. To use non-toxic concentrations for the host cells when assessing antiviral activity, cytotoxicity tests were performed beforehand on these cells (Martinez et al. 2021). Cellular viability (CV) was measured using the Neutral Red (NR) uptake assay, following a procedure previously described by Borenfreund and Puerner (1985). Different dilutions of each extract were added to a confluent monolayer of cells (1.04 mg ml^{-1}, 0.52 mg ml^{-1}, 0.26 mg ml^{-1}, and 0.13 mg ml^{-1}) (Pasquereau et al. 2021). Untreated MDBK cells were used as cellular control (CC) and Acyclovir was used as positive control. In the IC50 assay, MDBK cells were infected with CpHV-1 at a MOI of 0.1. The absorbance was measured at 540 nm. The percentage of CV (% CV) was calculated with respect to CC (100% CV) (Martinez et al. 2021). CC50 and IC50 were obtained by nonlinear regression fit using the Variable Slope Model, in GraphPad Prism version 5.00 for Windows (Graph-Pad Software, San Diego, CA, USA). The selectivity index was calculated based on the ratio of the 50% cytotoxic concentration and the 50% inhibitory concentration (CC50/IC50) (Pasquereau et al. 2021). The significant differences between groups (95% CI) for viral titration quantification were determined by One Way ANOVA followed by Bonferroni post-test in Graphpad Prism software.

b) Plaque size assay. MDBK cells were infected with CpHV-1 ($10^{7.5}$ DICT 50% ml^{-1}) at an M.O.I. of 0.1 and incubated at 37 ºC for 45 min. The infected cells were washed with MEM and the medium was replaced with 1 ml of semi-solid medium. Two days post-infection, cultures were fixed with 4% paraformaldehyde for 2 hours and stained with 1% crystal violet solution as described by Ladelfa et al. (2011) to compare the size of lysis plaques. Isolated and randomly chosen lysis plaques were photographed, and the ImageJ program was used to calculate their surfaces (ImageJ http://rsb.info.nih.gov/ij/). A comparison of means was carried out using the ANOVA test. Significant differences between means were determined by the Tukey comparison test (p values<0.05 were considered significant). A completely randomized design with five treatments was used to analyze the size of the lysis plaques.

c) Types of treatment and viral titration. A concentration of 0.52 mg ml^{-1} was chosen for all extracts, as it provided an optimal balance between cytotoxicity and the duration of action on viral replication.

d) Post-treatment with the compounds. MDBK cells were infected with CpHV-1 (10^7 DICT 50% ml^{-1}). After 45 minutes of adsorption at 37 ºC the cultures were treated with 1 ml of MEM containing *P. quadrangularis*, *L. divaricata* or *M. verticillata* at concentrations of 0.52 mg ml^{-1}, or a combination of the the extracts with or without aciclovir. After the 2-hour treatment, the monolayers were washed and supplemented with MEM.

Simultaneous Treatment (compound + virus). MDBK cells monolayers were infected simultaneously with 100 µl of a virus dilution containing 10^7 DICT 50% ml^{-1} with 0.52 mg ml^{-1} of *P. quadrangularis*, *L. divaricata* or *M. verticillata,* or a combination of the extracts with or without aciclovir. After 2 hs of incubation at 37 ºC, the cells were washed and supplemented with MEM.

Pre-Treatment with the compounds. MDBK cells were treated with 1 ml of MEM containing *P. quadrangularis*, *L. divaricata* or *M. verticillata* at concentrations of 0.52 mg ml^{-1}, or a combination of extracts with or without aciclovir. After the 2-hour treatment, the monolayers were washed with MEM and infected with CpHV-1 (10^7 DICT 50% ml^{-1}) for 45 minutes. Each assay included the following controls: non-infected and untreated cells (cellular control); non-infected cells treated with *P. quadrangularis*, *L. divaricata*, or *M. verticillata* (extract control); infected untreatment cells (virus control); and a positive antiviral control (acyclovir). The supernatants of infected cultures were harvested after 20 hours (Engels et al. 1983, 1987, Montagnaro et al. 2013), and the amount of intra and extracellular infectious virus was determined by viral titration on MDBK cells (Álvarez et al. 2009, Reed and Muench, 1938).

RESULTS

CC50, IC50 and SI. Table 1 summarizes the results of CC50, IC50 and SI. The extract of *L. divaricata* exhibited the lowest cytotoxicity with a CC50 value of 2.4 mg ml^{-1}, while the extracts of *P. quadrangularis* (2016) showed higher toxicity. Regarding antiviral activity, the extracts of *M. verticillata* and *P. quadrangularis* (2003) demonstrated the best antiviral activity with an IC50 = 0.16 mg ml^{-1}. The highest selectivity index was observed for *M. verticillata*, with a value of 10, indicating a better balance between antiviral efficacy and toxicity compared to the other extracts. *P. quadrangularis* (2016) showed relatively low SI values due to higher cytotoxicity, while *L. divaricata* displayed moderate cytotoxicity and antiviral activity. Acyclovir, used as a reference, exhibited a substantially higher SI, underscoring its established efficacy.

Table 1. Results of CC50, IC50 and SI for the extracts and acyclovir.

Extract	CC50 (mg ml^{-1})	IC50 (mg ml^{-1})	SI
L. divaricata	2.4	0.26	***9.2***
M. verticillata	1.6	0.16	***10***
***P. quadrangularis* (2016)**	0.57	0.32	1.78
***P. quadrangularis* (2003)**	0.9	0.16	5.6
Acyclovir	28	0.105	>200

The best SI are underlined and in bold.

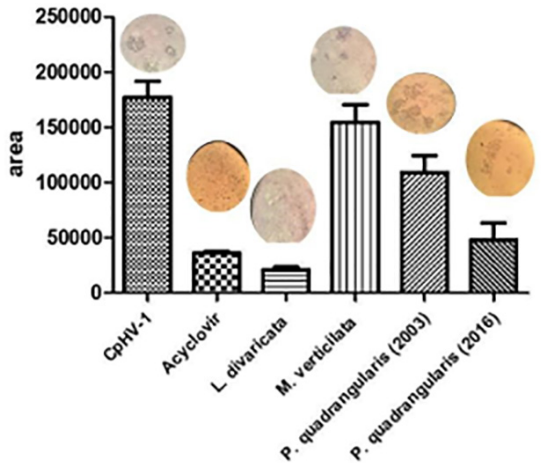

Figure 1. Area of lysis plaques expressed in pixels. Image of the lysis plaques after the different treatments.

An assay was performed to evaluate the antiviral activity of the extracts at 2 hours and 48 hours (data not shown), obtaining the same inhibition results. Considering its potential use in animals and the practicality of a single administration for therapeutic treatment, the following assays were conducted with this incubation time of the extracts on the previously infected monolayers.

Plaque size assay. Significant differences ($p<0.05$) were observed between the sizes of the lysis plaques of the antiviral treatments evaluated (Figure 1). All the extracts reduced plaque lysis to different degrees, with *L. divaricata* and *P. quadrangularis* (2016) being the most effective. *L. divaricata* was as efficient in reducing plaque lysis as the positive control Acyclovir**.**

Quantification by viral titration. The greatest inhibition of viral replication was observed in both the intracellular and extracellular fractions when monolayers were treated with *L. divaricata* during pretreatment and simultaneous treatment (Figure 2). When it was used after infection (post treatment), a greater reduction of the viral titer was noted in the extracellular fraction treated with *L. divaricata* compared to Acyclovir (Figure 2).

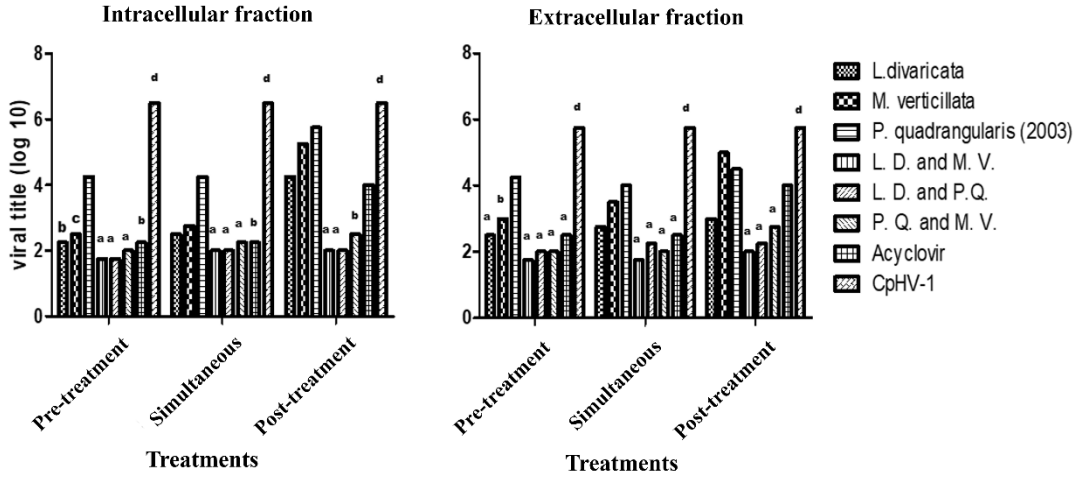

Figure 2. Viral titers (log10) of the intracellular and extracellular fraction obtained at 20 hours after the different treatments. The letters indicate significant differences ($p<0.05$) as follows: a: ***, b: ** and c* compared to the CpHV-1 control. L.D: *L. divaricata,* M.V.: *M. verticillata*, P.Q., *P. quadrangularis*.

With the extract combinations, a marked reduction in both fractions of the viral titers was seen with all the treatments and all the combinations, especially with *L. divaricata* and *M. verticillata* (Figure 2). All treatments showed significant differences ($p<0.05$) with the control without treatment, but the treatments did not show significant differences between them.

DISCUSSION

The failure of drugs and the development of resistance have led to a growing interest in natural products, especially plants, and research into antiviral agent discovery. Many studies have been carried out over the years to isolate bioactive antiviral compounds from plants.

New prophylactic measures must take into account not only the prevention of the clinical consequences of an infection caused by viruses, but also the decrease in titers and the duration of viral excretion into the environment for the prevention of further infections. Extracts from *L. divaricata, M. verticillata* and *P. quadrangularis* were used in this assay, at concentrations of 0.52 mg ml^{-1} in three formats of treatments: pre-treatment with the compounds, simultaneous (compound + virus) and post-treatment with the compounds. The greatest viral inhibition (at least a decrease of 35%) was observed in pre-treatment and simultaneous treatment with the *L. divaricata* extract.

The use of *L. divaricata* as a medicine by indigenous peoples of southwestern Argentina is well-documented, particularly for treating venereal diseases, gastric disturbances, respiratory inflammations, tuberculosis, and as an antiseptic, expectorant, and stimulant (Alonso 2023). Acute and subacute toxicity studies with *L. divaricata* in mice reported that it does not produce behavioral changes or tissue damage in histological examinations, nor any obvious signs in clinical evaluations of the animals (Bligliani et al. 2010). In other studies, it was shown to be harmless with an LD50 (median lethal dose) of 4000 mg

kg^{-1} for females and 10000 mg kg^{-1} for male mice (Anesini et al. 1997) and was also non-irritating in topical toxicity tests using the rabbit skin irritation model, such as the Draize test and acute ocular irritation test in rabbits (for 7 days), conducted on male New Zealand White (NZW) rabbits (Peralta et al. 2015).

It has been shown to demonstrate *in vitro* antioxidant, antiinflammatory and immunomodulator through the use of the aqueous extract of *L. divaricata* (Peralta et al. 2022, Alonso et al. 2023). The selectivity index obtained in this work for the extracts of *L. divaricata* (9.2) and *P. quadrangularis* (5.6) is comparable to those reported for *Guajillo* and *Ancho* against Herpes simplex type 1 (Ordaz-Trinidad and Dorantes-Álvarez 2018).

In all treatments formats, whether applied individually or in combination with *L. divaricata*, a reduction in viral titer was observed through quantification by limiting dilution in the intra and extracellular fractions titrated at 20 hours with at least a 35% decrease in the intracellular fraction and a 50% decrease in the extracelular fraction compared to the control without treatment. This is similar to *Larrea tridentate* which shows antiviral activity against varicella zoster virus (HHV-3), this extract contains NDGA (Nordihydroguaiaretic acid) in the leaves, constituting approximately 50%. Within plant families, active molecules are often present in similar proportions. Since the extract contains NDGA, the most important active compound known to inhibit 5-lipoxygenase, this compound could be involved in the eosinophilia induced by the extract (Peralta et al. 2015). In infections with CpHV-1, varying degrees of inflammatory lesions with eosinophilic and amphophilic intranuclear inclusion bodies have been reported (Candanosa et al. 2016). Given that NDGA has proven anti-inflammatory properties, it could be effective in counteracting the inflammation caused *in vivo* in goats. Therefore, it may be responsible for the inhibition of viral replication observed by the extract in this work. A study demonstrated that NDGA inhibits *in vivo* transcription from the HSV (herpes simplex virus) ICP4 promoter, a gene essential for HSV replication and one of the first genes expressed in the HSV lytic transcriptional program (Chen et al. 1998). Similar mechanisms of action may play a role against CpHV-1 infections, as evidenced by the observed inhibition of viral replication. Additionally, it prevents the binding of the eukaryotic transcription factor, Sp1, to its cognate binding sites on the HIV promoter, thus indicating a likely mechanism for transcriptional inhibition (Chen et al. 1998). Similar mechanisms of action could explain the effects observed against CpHV-1 infections, such as the inhibition of viral replication observed in this work. This may define the probable gene target of the anti-HSV activity of the compound. It was also reported in the inhibition of Junín virus (Konigheim et al. 2006) and Dengue virus (Soto-Acosta et al. 2014). Later studies showed that NDGA reduces the frequency of HSV reactivation from explanted latently infected mouse trigeminal ganglia (Park et al. 2003). Therefore, the importance of controlling the infection can be stated.

M. verticillata exhibited the highest viral inhibition in both pretreatment and simultaneous treatment, similar to the results observed by Schuhmacher et al. (2003) in an assay with *Mentha piperita* against herpes simplex virus. The inhibitory activity against herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) was tested *in vitro* on RC-37 cells using a plaque reduction assay (Schuhmacher et al. 2003, Saz et al. 2012). These results indicate that *M. verticillata* affected the virus before adsorption, but not after penetration into the host cell.

M. verticillata reduces inflammatory biomarkers and histological damage in an experimental model of colitis (Rodríguez Basso et al. 2021). It contains essential oil and flavonoids in the leaves, which give it aromatizing, antiseptic and tranquilizing properties (Soria and Ramos 2015). The *in vitro* antiviral activity of the essential oil from *M. verticillata* (Mv-EO) was investigated against herpes simplex virus type 1 (HSV-1) and pseudorabies virus (PrV), demonstrating its antiviral capacity by inhibition of multiplication (Primo et al. 2001, Vogt et al. 2010). Numerous investigations have highlighted the anti-allergic activities of *Lamiaceae* species with their active principles and crude extracts (Sim 2019). It has been reported that with a dose of 0.04% Mv-EO in water, equivalent to an average daily intake of 35 mg kg^{-1} bw/day, the animals remained healthy, with no signs of toxicity or mortality observed throughout the 45-day study, indicating that Mv-EO did not cause any genotoxicity on its own (Escobar et al. 2019). Pulegone was found to be present in the essential oil, and results suggested that this compound is effective in the inhibition of β-hexosaminidase (glycolytic enzyme) release from human basophils (Cariddi et al. 2007, Sim 2019). Other studies have shown that in serum from healthy individuals, the oil (0.8 and 0.16 mg ml^{-1}) increased IFN-γ levels compared to cultures without stimulation. A recent review (Hilfiger et al. 2021), lists several terpenes that modulate the activity of cytokines, including the tumour necrosis factor (TNF-α), a cytokine involved in the primary onset of inflammatory responses its maintenance, and chronicity (Basbaum et al. 2009). Menthol and pulegone are cited in this list. He et al. (2013) reported the effect of pulegone against influenza virus A/PR/8/34 (H1N1). *In vivo* experiments showed that pulegone (0.19 mg kg^{-1}) had a significant effect on decreasing serum levels of IL-6; and TNF-alpha. The *in vitro* experiment showed that the expression levels of IRAK4 mRNA and IFN-beta were significantly increased in pulegone (0.1 g L^{-1}) groups. Administration with pulegone therapeutically (but not preventively) can significantly decrease the hemagglutination titer. The *in vivo* antiviral mechanism is related to the regulation of IFN-alpha, IFN-beta and IL-2. The results obtained in this test suggest that essential oil, for possible *in vivo* applications, would harness the physiological regulations associated with IFN-γ such as the reduction of allergic symptoms, increased antiviral capacity antiviral and enhanced anti-cancer potential (Cariddi et al. 2007). A recent review (Quintans et al. 2019) also lists several terpenes that modulate the activity of cytokines, including the tumour necrosis factor (TNF-α), a cytokine involved in the primary onset of inflammatory responses, its maintenance and chronicity (Basbaum et al. 2009) and menthol and pulegone are cited in this list.

P. quadrangularis has numerous applications and has been traditionally used in folk medicine to treat

headaches. Other studies showed that it could be used as a gastroprotective agent because it is rich in phenolic compounds and terpenoids and thus can be useful for the preparation of nutritional supplements (Ardiles et al. 2018). *P. quadrangularis* exhibits moderate antimicrobial activity against various clinical isolates of *Helicobacter pylori* (Helwig et al. 2018). The antibacterial activity of different species of *P. quadrangularis* against sensitive and multiresistant Gram-positive and Gram-negative bacteria such as *Enterobacter cloacae*, *Pseudomona aeruginosa* and *Proteus mirabilis* has been reported (Zampini et al. 2009); and *P. lepidophylla* has been studied for the control of phytopathogenic fungi of *Penicillium digitatum* (Palavecino et al. 2016) as well as for its antifungal activity of *P. quadrangularis* extracts against *Fusarium verticillioides* (Di Ciaccio et al. 2018).

On the other hand, all possible combinations were made with the three extracts to evaluate synergism. When using the extracts as synergists, in all three treatments that contained *L. divaricata* there was a notable reduction in viral titers, which may also be responsible for inhibiting the release of the virus from the cell. This was also seen in a study where the extracts *Equinacea purpurea* and *Myrciaria dubia* acting together produced an even greater inhibition of the influenza virus (Vivanco Roberto 2012). It should be noted that when the extracts and acyclovir were tested separately at the same dose that was used in combination of extracts, no antiviral activity was observed.

The results showed that the treatments mainly with *L. divaricata*, and formulations where it participated, were potent in reducing the replication of CpHV-1 *in vitro,* evaluated in the different activities tested, thus supporting the bases and validating the traditional uses as antivirals. This promotes further studies in search of the compounds responsible for these bioactivities and their establishment as candidates for the treatment and prevention of CpH V-1 in goats. In this context, there is a need to further investigate plant extracts by identifying their bioactive metabolites. In future studies, it is proposed to carry out the *in vitro* and *in vivo* characterization of the active principles of *L. divaricata* (nordihydroguayaretico), *M. verticillata* (pulegone) and p-coumaroyloxytremetone from *P. quadrangularis*. These findings open several perspectives in terms of future studies and therapeutic possibilities for animal herpesviruses (Lanave et al. 2019).

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