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# Chemical composition and antiviral activity of Venezuelan *Pimenta racemosa* essential oil and its major component (Eugenol) against Oropouche Virus

## Composición química y actividad antiviral del aceite esencial de *Pimenta racemosa* venezolana y de su componente mayoritario (Eugenol) contra el virus Oropouche

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**Abstract**

The chemical composition of the essential oil of *Pimenta racemosa* from one sample collected in Caracas, Venezuela, was determined by GC-MS. A high yield of leaf oil (1.5%) was obtained by steam distillation, yielding a volatile mixture rich in phenylpropanoids, with eugenol as the major compound. Twelve components were identified, the major ones being eugenol (78.04%), chavicol (10.05%), β-myrcene (5.67%), and linalool (2.30%). There is a growing interest in exploring the potential biological activities of essential oils. In this context, the essential oil (EO) of P. *racemosa* and its major compound, eugenol, were evaluated for antiviral activity against the Oropuche (OROV) virus. The results of the antiviral evaluation indicated modest, dose-dependent antiviral activity.

**Keywords**: Antiviral, Oropuche, *Pimenta racemosa*, Eugenol, Venezuela

**Resumen**

La composición química del aceite esencial obtenido a partir de una muestra de *Pimenta racemosa* colectada en Caracas, Venezuela fue determinada mediante CG-EM. Mediante la técnica de arrastre con vapor, se obtuvo aceite de hojas frescas con un alto rendimiento (1,5%). Esta mezcla de volátiles fue rica en fenilpropanoides, con el eugenol como compuesto más abundante. Un total de doce compuestos fueron identificados, siendo los compuestos mayoritarios eugenol (78,04%), chavicol (10,05%), β-mirceno (5,67%) y linalool (2,30%). Existe un creciente interés en explorar las posibles actividades biológicas de los aceites esenciales. En este contexto, se evaluó la actividad antiviral del aceite esencial (AE) de *P. racemosa* y, en particular, la del eugenol, su principal componente, frente al virus Oropuche (OROV). Los resultados de la actividad antiviral indicaron una acción modesta, dependiente de la dosis.

**Palabras clave**: Antiviral, Oropuche, *Pimenta racemosa*, eugenol, Venezuela

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## Introduction

The Myrtaceae family comprises a vast range of aromatic plants with well-established medicinal properties, encompassing approximately 150 genera and over 5,900 reported species (Grattapaglia et al., 2012). This family is primarily distributed throughout the Southern Hemisphere, particularly in tropical regions and Australia. Several species within this family are widely recognized, including *Eucalyptus camaldulensis, Syzygium aromaticum, Pimenta dioica, Psidium guajava,* and *Myrtus communis* (Wilson, 2011). Notably, the Caribbean islands and northern South America are exceptionally rich in the diversity of genera in this family.

The genus *Pimenta*, a group of flowering plants within the Myrtaceae, comprises 15 known species, the majority of which are native to the Caribbean region (Wilson, 2011). Various biological activities have been reported for this genus, including antioxidant, antimicrobial, antihypertensive, hypoglycemic, anticancer, anti-inflammatory, analgesic, antipyretic, acaricidal, anxiolytic, and antiviral properties. Among these, *Pimenta dioica* and *Pimenta racemosa* are the most prominent, particularly in traditional medicine (El-Nashar et al., 2023). In general, the *Pimenta* genus is considered to possess significant pharmacological potential (Reis et al., 2010).

*Pimenta racemosa* var. *racemosa* (M. Miller) J.W. Moore is native to Venezuela, Guyana, and the Caribbean islands (Aristiguieta, 1973). Various common names, such as malagueta, bay-rum, and pimienta, are known. The species has spread to other countries and continents (Pragadheesh et al., 2013), where it is valued for the pleasant aroma of its essential oil (EO) and its proven medicinal properties, including antibacterial (Contreras-Moreno et al., 2017; Ayoub et al., 2022), antifungal (Junheon et al., 2008), antiviral (Elshaarawy et al., 2024), and antioxidant activities (Contreras-Moreno et al., 2017; Eldahsan et al., 2025). Furthermore, *P. racemosa* is highly regarded in the perfumery industry due to the unique fragrance of its EO. While the chemical composition may vary in concentration, the primary constituents are typically derivatives of the phenylpropanoid eugenol. Beyond its EOs, the antinociceptive and anti-inflammatory effects of its extracts and isolated metabolites have been documented (Fernández et al., 2001; Garcia et al., 2004).

Essential oils from medicinal plants exhibit a broad spectrum of pharmacological activities. Lately, their evaluation as antiviral agents has increased significantly. These activities can be mediated by individual compounds or through the synergistic action of various constituents within the oil's complex chemical matrix. Utilizing EOs to combat viral infections represents a promising avenue for developing new treatments and is a rapidly expanding field of study (Boukhaten, 2020; Ojah, 2020; Baker et al., 2021; Anjie et al., 2025; Castellanos-Huerta et al., 2025). For instance, a recent study demonstrated that *Pimenta racemosa* essential oil exhibits significant activity against HSV-1 and HSV-2 (Elsharaawy et al., 2024).

Eugenol, a key phenylpropanoid found in the EOs of many plant species, is recognized for its analgesic, antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Anuj and Anjay, 2010; Nissar et al., 2021; Ulanowska and Olas, 2021). Its antiviral potential has also been the subject of numerous investigations (Benencia and

Courréges, 2000; Lane et al., 2020; Paidi et al., 2021; Rizutti et al., 2021; Kaushik et al., 2023; Chen et al., 2025; Wang et al., 2022, 2024, 2025).

In recent years, viral infections have garnered global attention as leading causes of mortality, a reality starkly exemplified by the COVID-19 pandemic. While SARS-CoV-2 continues to be extensively researched in the pursuit of alternative treatments, many other viral pathogens remain poorly understood. The diseases caused by these viruses are often classified as neglected because they primarily affect populations in localized geographic areas or regions with limited access to healthcare. A poignant example is the Oropouche virus (OROV) (*Orthobunyavirus oropoucheense*). Although OROV has been detected in several Central and South American countries, its clinical presentation closely resembles that of common arboviral diseases such as dengue, Zika, and chikungunya, leading to substantial underestimation of reported cases (Tilston-Lunel, 2024; Zhang et al., 2024; Bai et al., 2025).

In an ongoing project in our laboratory, we are evaluating the chemical composition and antiviral activity of essential oils derived from plants used in Venezuelan traditional medicine, and, due to its wide distribution across the country, *Pimenta racemosa* was selected to investigate the chemical profile and antiviral efficacy of its essential oil and its major component, eugenol, against the Oropuche virus (OROV).

## Material and Methods

*Plant material:* The leaves of *Pimenta racemosa* were collected in Caracas, Distrito Capital, in June 2025. The Botanist Giovanina Orsini identified the species, and a voucher specimen, number MYF-23936, was deposited in the Víctor Manuel Ovalles Herbarium of the Facultad de Farmacia, Universidad Central de Venezuela.

*Oil isolation:* Fresh leaves (186g) were steam distilled for 3 h using a Clevenger-type apparatus. This procedure was repeated 3 times to obtain a yellow oil with an average yield of 1.5%. The obtained EO was dried over anhydrous $Na_2SO_4$ and stored in an amber vial at -5 °C until used for chemical and biological analysis.

### Identification of the Chemical Constituents of the Essential Oil

For the qualitative analysis, an Agilent gas chromatograph model 8860 (Agilent Technologies, Santa Clara, CA, USA) was used, coupled to a mass spectrometer (quadrupole) detector (MS) model Agilent 5977 (Agilent Technologies, Santa Clara, CA, USA). The GC-MS analyses were performed using an Agilent HP-5MS fused silica capillary column (30 m x 0.25 $\mu$m film thickness x 0.250 mm i.d.) for the separation. The samples (1$\mu$L) were injected with a split ratio of 20:1. Hydrogen was used as the carrier gas at a constant flow rate of 1.2 mL/min (average speed of 34 cm/s). The injector temperature was set at 250 ºC. The oven temperature program was initiated at 40 °C (held for 2 min), increased to 140 °C (held for 5 min) at 10 °C/min, and finally raised to 240 °C (held for 5 min) at 5 °C/min.

The operating conditions for the MSD were as follows: an electron ionization energy of 70 eV, a mass range of 40–550 *m/z*, and a scan rate of 2 scans/s. The essential

oil was diluted to 1% in chloroform, and a 1 $\mu$l volume was injected. Data for *n*-alkane standards ($C_8$ to $C_{20}$) were also collected under the same method for linear retention index (LRI) determinations.

Identification of oil components was achieved based on LRIs (determined with reference to the homologous series of *n*-alkanes) and by comparing their fragmentation patterns in the mass spectrum with those reported in the literature (Adams, 2007) and stored in the NIST 23 and Wiley 1998 mass spectral libraries. Data analysis was conducted using MassHunter Workstation Software Version 10.0 (Agilent Technologies, Santa Clara, CA, USA).

LRIs were determined using the series of $C_8$-$C_{20}$ *n*-alkanes, processed under the same chromatographic conditions as the sample, according to the following equation:

$$\mathbf{LRI} = \mathbf{100} * \left( \mathbf{y} + \frac{t_{R(x)} - t_{R(y)}}{t_{R(z)} - t_{R(y)}} \right)$$

where:

$t_{R(x)}$ = Retention time of the target compound.

$t_{R(y)}$ = Retention time of the *n*-alkane eluting immediately before the target compound.

$t_{R(z)}$ = Retention time of the *n*-alkane eluting immediately after the target compound.

y = Carbon number of the *n*-alkane eluting immediately before the target compound.

Compound concentrations were calculated from their peak areas in the chromatogram.

## Biological activity

### Cytotoxicity Assays

To evaluate the cytotoxic effect of the EO, Vero E6 cells (20,000 cells/well) were plated overnight and then exposed to two concentrations of the compound. All essential oils and compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mg/mL, and dilutions were prepared in culture medium. The tested concentrations (5 and 10 $\mu$g/mL per well) were assessed 24 hours post-exposure using the MTT method (Mossman, 1983). The assay is based on the reduction of MTT, a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), into formazan, an insoluble purple crystal. Compounds exhibiting cytotoxicity >20% were discarded.

## Antiviral Activity Assay

### Quantification of Oropouche viral particles under treatment with P.r EO and eugenol

For viral particle quantification using plaque-forming unit (PFU) assay, approximately 200,000 Vero E6 cells were incubated in each well of 24 plates in a final volume of 500 $\mu$L of RPMI medium supplemented with the virus, yielding approximately 50 plates per well. Each oil was added at concentrations ranging from 0.5 $\mu$g/mL to 5 $\mu$g/mL for 1 h. Subsequently, each well was washed twice with RPMI medium, and the oils were reintroduced at the same initial concentration in their respective wells, along with RPMI medium and 1.50% carboxymethylcellulose. As a

negative control for infection, Vero E6 cells were treated with RPMI medium alone; as a positive control, cells exposed to the virus were treated with RPMI medium without oil. After 72 h, 500 $\mu$L of 4% formaldehyde was added to each well, and the following day, each well was washed three times with distilled water. Subsequently, 100 $\mu$L of 1% crystal violet was added to each well for 15 minutes, the excess was washed off, and the next day, the plaque-forming units were counted using a stereomicroscope (Nikon, SMZ745T) and a halogen lamp (Nikon, NI-150). The number of plaques obtained was compared with those from the positive and negative infection controls, and the assays were performed in triplicate.

## Results

### Qualitative and Quantitative Chemical Composition of the Essential Oil of *Pimenta racemosa*

The leaves of *Pimenta racemosa* gave, after three distillations, an average yield of 1.5% by weight of fresh material. An intense and pleasant aroma characterized the yellow oil.

The qualitative and quantitative composition of the EO was established by gas chromatography-mass spectrometry (GC-MS). The chemical profile of the volatile compounds was determined by comparing their electron mass spectra with those stored in the mass spectrometer libraries (NIST 23, Wiley 98), as well as by comparing their linear retention indices with reported values in the literature.

A total of twelve compounds were detected, identified, and quantified. The essential oil composition was dominated by the presence of phenylpropanoid compounds (88.89%), followed by monoterpene hydrocarbons (6.95%) and oxygenated monoterpenes (2.68%). The presence of saturated alcohols and ketones in minor amounts, together with unsaturated alcohols, comprises the compounds detected in the EO, which achieved 99.9% identification. The total number of components is presented in Table I, and the chromatogram showing the chemical structures of the main compounds is shown in Figure 1.

### Antiviral activity

The EO and eugenol were evaluated at subcytotoxic concentrations ranging from 0.5 $\mu$g/mL to 5.0 $\mu$g/mL to elucidate the dose-response. Controls were untreated cells. As shown in Figures 2 and 3, the results indicate a dose-dependent decrease in the production of OROV particles with increasing concentrations of *P. racemosa* essential oil and eugenol. These similar results for the EO and the compound suggested that the activity is due to the major compound.

## Discussion

The composition of *Pimenta racemosa* essential oil has been reported from various regions worldwide, including India, Benin, Ecuador, Cuba, the Dominican Republic, Egypt, Jamaica, and Venezuela. The literature on this research shows that the chemical composition of the essential oil of this renowned species, as with other plant species, depends on geographic and climatic conditions. The composition in different places is sometimes richest in the number of compounds, but in general, the

**Table I.**
Chemical components of *Pimenta racemosa* essential oil

| Peak | Rt(min) | Compound | % | RI | RI Ref | Type | MF |
|---|---|---|---|---|---|---|---|
| 1 | 9.849 | 1-octen-3-ol | 0.45 | 987 | 974 | UAA | $C_8H_{16}O$ |
| 2 | 10.105 | 3-octanone | 0.75 | 994 | 979 | SAK | $C_8H_{16}O$ |
| 3 | 10.245 | β-Myrcene | 5.67 | 998 | 988 | MH | $C_{10}H_{16}$ |
| 4 | 10.472 | 3-octanol | 0.27 | 1004 | 988 | SAA | $C_8H_{18}O$ |
| 5 | 11.411 | *p*-Cymene | 0.24 | 1021 | 1020 | MH | $C_{10}H_{14}$ |
| 6 | 11.551 | *D*-Limonene | 0.85 | 1024 | 1024 | MH | $C_{10}H_{16}$ |
| 7 | 12.471 | (*E*)-β-Ocimene | 0.19 | 1048 | 1044 | MH | $C_{10}H_{16}$ |
| 8 | 14.430 | Linalool | 2.30 | 1099 | 1095 | OM | $C_{10}H_{18}O$ |
| 9 | 17.146 | Terpinen-4-ol | 0.38 | 1173 | 1174 | OM | $C_{10}H_{18}O$ |
| 10 | 20.060 | Chavicol | 10.05 | 1255 | 1247 | PP | $C_9H_{10}O$ |
| 11 | 23.517 | Eugenol | 78.04 | 1359 | 1356 | PP | $C_{10}H_{12}O_2$ |
| 12 | 24.997 | Methyl Eugenol | 0.80 | 1405 | 1403 | PP | $C_{11}H_{14}O_2$ |

| | |
|---|---|
| Monoterpenes hydrocarbons (MH) | 6.95 |
| Oxigenated monoterpenes (OM) | 2.68 |
| Phenylpropanoids (PP) | 88.89 |
| Unsaturated aliphatic alcohols (UAA) | 0.45 |
| Saturated aliphatic ketone (SAK) | 0.75 |
| Saturated aliphatic alcohol (SAA) | 0.27 |
| Total identification | 99.99 |

Rt: Retention time; RI: Retention index; RI Ref.: retention index reference; MF: Molecular formula

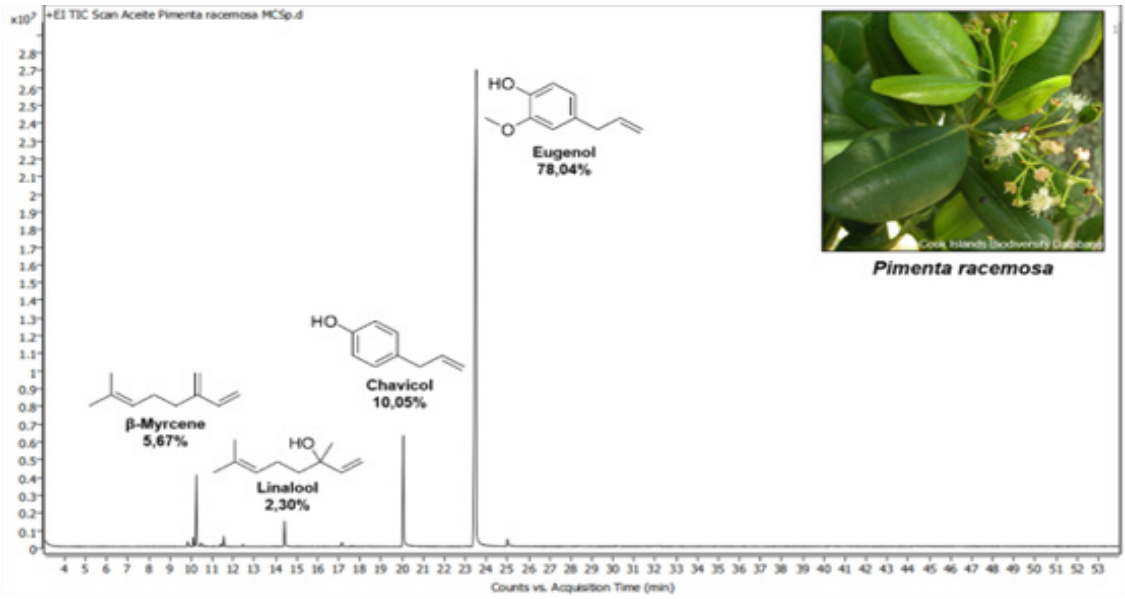


**Figure 1.**
GC-MS profile of *Pimenta racemosa*, showing the structures of the major compounds

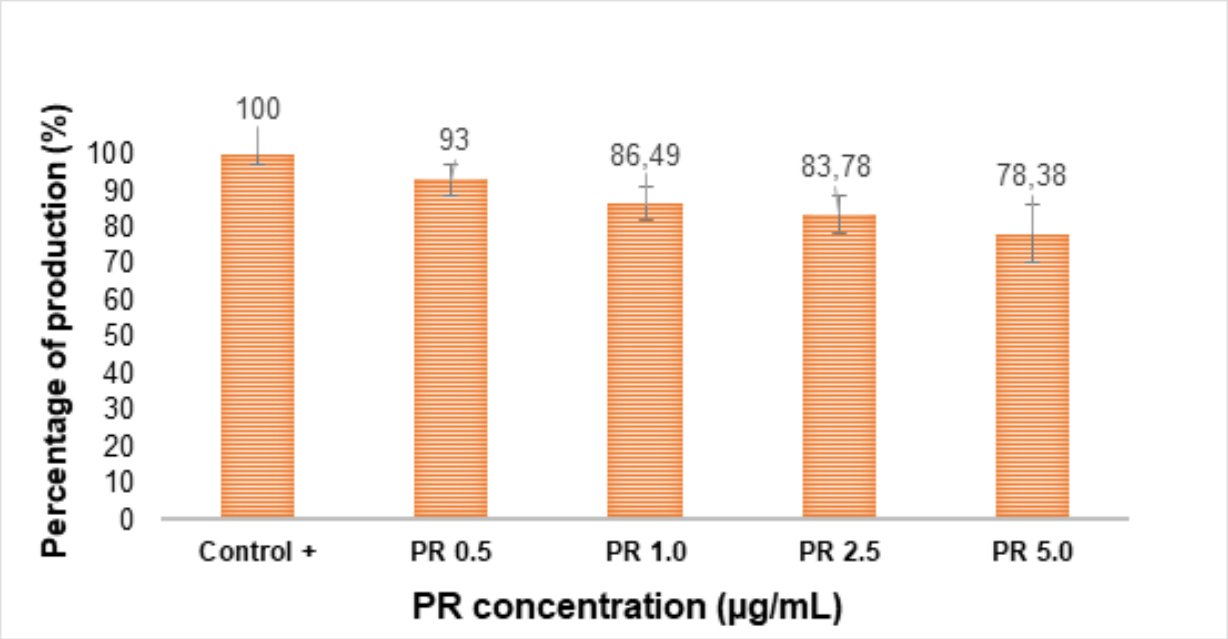


**Figure 2.**
Viral particle production under *P. racemosa* EO treatment

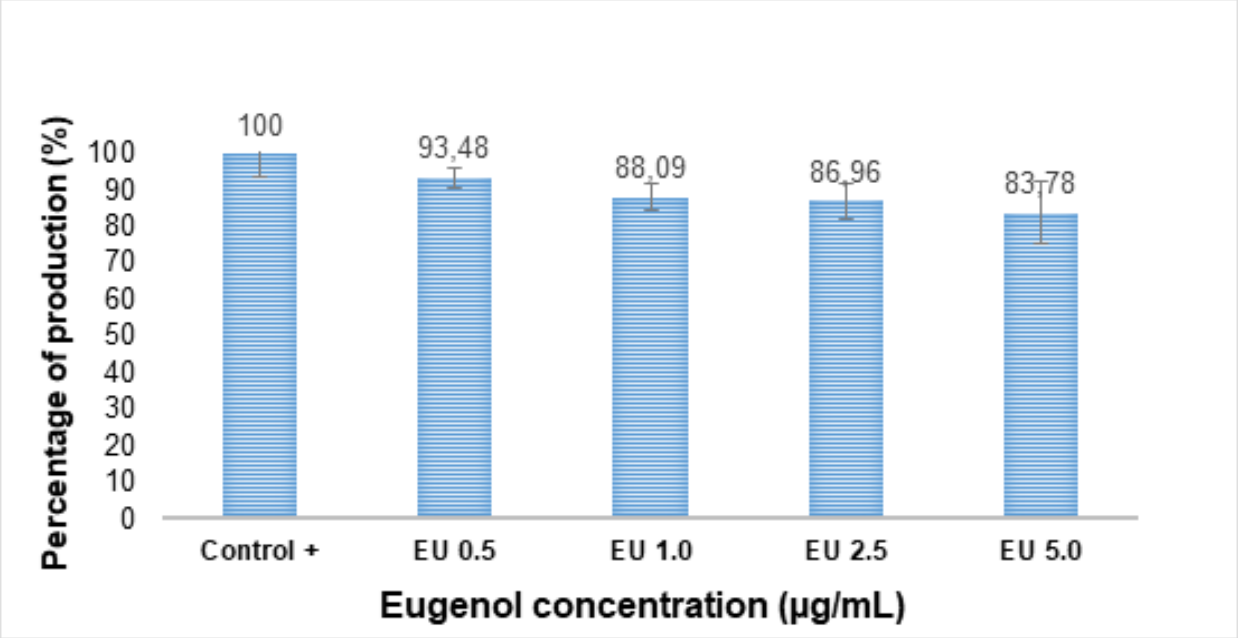


**Figure 3.**
Viral particle production under eugenol treatment

major compounds, with some exceptions, are the same; differences in the composition and contents of natural essential oils are common. Many factors influence the differences, such as environmental conditions, plant organ, climate, vegetative stage, and also the procedure used to obtain the oil. It is interesting to consider these differences because, in terms of bioactivity, the major components of the essential oils are considered responsible for the activity; however, minor compounds can produce synergistic or antagonistic effects that could influence the overall outcome.

Phenylpropanoids are the most common compounds found in *Pimenta racemosa*. Studies from Egypt (Ayoub et al., 2022; Eldashan et al., 2025), India (Pragadesh et al., 2013), Benin (Alitonou et al., 2012), and Venezuela (Contreras-Moreno et al., 2014, 2017) have identified eugenol as the predominant constituent. However, concentration and secondary chemical profiles vary. The oil compositions reported from other places, such as Ecuador (Bustamante et al., 2017) and Cuba (Bello et al., 1995, 1998), differ, with other compounds reported at higher concentrations. Other components, such as β-myrcene, chavicol, limonene, and linalool, are common in all these oils. In the present study, we report an eugenol concentration of 78.04%, which, to the best of our knowledge, is the highest recorded for the species. This sample collected in Caracas significantly exceeds the eugenol levels previously reported for the Venezuela Andes region (Contreras-Moreno et al., 2014, 2017). This exceptionally high concentration suggests that eugenol plays a defining role in any biological activity attributed to this oil.

Regarding the results on the inhibition of Oropuche virus, we initially presume that this oil is a good candidate for evaluation due to its high eugenol content. This small compound has been reported to have good results in assays against viruses such as Ebola, SARS-CoV-2, influenza A virus, and hepatitis (Chen et al., 2025), as well as Largemouth bass ranavirus (LMBV) (Wang et al., 2025), but in our assays on Oropuche (OROV), it is low. Each microorganism is distinct, and we demonstrate that neither the oil mixture nor the pure compound is active against OROV.

## Conclusions

In conclusion, the steam distillation of fresh *P. Racemosa* leaves yielded an essential oil (1.5% w/w), representing the highest yield reported to date for this species. Characterized by a pale yellow color and a pleasant odor, the oil's chemical profile is dominated by phenylpropanoids, chiefly eugenol (78.04%), along with chavicol (10.05%), β-myrcene (5.67%), and linalool (2.30%). This study represents the first evaluation of the antiviral activity of *P. racemosa* essential oil and its major constituent, eugenol, against OROV, demonstrating that eugenol is responsible for the moderate viral inhibition observed. Ultimately, these findings expand our understanding of the biological potential of *Pimenta racemosa* essential oil.

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## Conflicts of interest

The authors declare that they have no known competing financial or personal relations that could influence the research reported in this work.

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