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Evaluation of the Antiviral Activity of Andiroba Oil (*Carapa guianensis* Aubl.) In an *In Vitro* Model Of HSV-1 Infection



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Abstract

Herpes simplex virus type 1 (HSV-1) is a highly prevalent pathogen associated with recurrent infections, highlighting the need for alternative antiviral agents. This study aimed to evaluate the antiviral activity of andiroba oil (*Carapa guianensis* Aubl.) against HSV-1 in vitro. Vero E6 cells were infected with HSV-1 and treated with andiroba oil at concentrations of 10 and 100 ng/mL. Antiviral activity was assessed by reduction of cytopathic effect (CPE), quantified through image analysis. Treatment with andiroba oil resulted in a reduction of virus-induced cytopathic effects, with a more pronounced effect observed at the higher concentration (100 ng/mL) and at greater viral dilutions. The data suggest a concentration-dependent trend; however, variability between replicates and the absence of molecular assays limit the robustness of the findings. These results indicate that andiroba oil may exhibit antiviral activity against HSV-1 in vitro. Further studies using standardized quantitative assays and molecular approaches are necessary to confirm these effects and clarify the underlying mechanisms.

Keywords: *Carapa guianensis*; Herpes simplex; Phytotherapy; Antivirals; Medicinal plants.

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INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is a double-stranded DNA virus responsible for widespread infections, particularly orofacial lesions, affecting a significant proportion of the global population. The virus is characterized by its ability to establish latency and cause recurrent infections, posing a continuous public health challenge (Zupin et al., 2018; Diefenbach & Fraefel, 2020). Current therapeutic approaches rely mainly on nucleoside analogs such as acyclovir, penciclovir, and valacyclovir. Although effective, these drugs require activation by viral and host enzymes, and their repeated use has led to the emergence of resistant viral strains. Additionally, their efficacy may be reduced in immunocompromised individuals, highlighting the need for alternative antiviral strategies (Looker et al., 2015; James et al., 2020). Natural products have gained attention as potential sources of new antiviral agents due to their chemical diversity and ability to act at different stages of the viral replication cycle. Among these, plant-derived compounds have shown promising biological activities, including antiviral effects (Jiang et al., 2016; Newman & Cragg, 2016; Akran et al., 2018; Steiner & Benninger, 2018; Moya et al., 2020; Gabaglio et al., 2021; Li et al., 2022; Pennisi et al., 2023; Kuo et al., 2023; Sem & Eroğul, 2024). Carapa guianensis Aubl., commonly known as andiroba, is a neotropical species widely distributed in the Amazon region. The oil extracted from its seeds has been traditionally used for its anti-inflammatory, analgesic, and insect-repellent properties. These biological activities are associated with its chemical composition, which includes fatty acids, glycerides, and limonoids, compounds that may exhibit pharmacological potential (Silva & Almeida, 2014; Meccia et al., 2013; Jesus et al., 2017; Araújo-Lima et al., 2018; Oliveira et al., 2018; Inoue et al., 2018; Moya et al., 2020; Soares et al., 2021; Amorim et al., 2022; Soares et al., 2021; Amorim et al., 2022). Despite its known bioactive properties, the antiviral potential of andiroba oil against HSV-1 remains poorly explored, particularly in controlled in vitro models. Therefore, this study aimed to evaluate the antiviral activity of Carapa guianensis oil against HSV-1 infection in vitro.

Methods and Methods

Acquisition of andiroba oil

The andiroba oil (AO) used in the study was sourced from a private company in Belém, Brazil, which provided a certificate of oil purity, as well as information about location, climate, soil type, and other characteristics crucial for quality assessment. The andiroba samples were collected in the northeastern region of Pará State, Brazil (coordinates: 1°43'04.0"S, 48°52'58.0"W). High-Performance Liquid Chromatography (HPLC) provided the composition of andiroba oil in denser molecules, which are not detectable through gas chromatography, enabling an understanding of which substances in andiroba contribute to its efficacy or lack thereof in the proposed treatment. For this purpose, analyses were performed using the Waters Breeze system equipped with a Waters 1525

binary HPLC pump and a Waters 2489 UV/VIS detection system. A SupelcoNucleosil® C18 column (250 x 3.2 mm, 5 µm) was used, with a flow rate of 0.5 mL/min, UV detection at 275 nm, and isocratic elution (H₂O-MeOH 53:47). Quantification of the andiroba oil was conducted using the software installed in the program, which provided the specific pure concentration of the compound.

Gas chromatography analysis (GC-MS)

The oil was analyzed through GC-MS, using a gas chromatograph (Varian CP 3380 model) equipped with an ion detector and a CP-Sil 88 capillary column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 µm; Varian Inc., USA). This step aimed to convert the fatty acids in the oil into methyl ester acids. The Varian Star 3.4.1 software was used for fatty acid quantification, generating chromatograms and mixtures of standard fatty acids (Nu-check-prep, Inc., USA). Fatty acid values were quantified as relative percentages of total acids.

Cell line and virus strain

VERO E6 cells, which are commercial continuous cultures of fibroblasts derived from the kidneys of the African green monkey (*Chlorocebus sabaeus*), were used. These cells were chosen because they are permissive to herpesvirus infection. A viral strain of HSV-1 (sample 2033-001561) was also used.

Cell line maintenance and expansion and preparation of viral stock suspensions

For maintenance and expansion, the cells were thawed and centrifuged at 1,500 rpm for 5 minutes. The medium was discarded, and the pellet was resuspended in 5 mL of Dulbecco's Modified Eagle Medium (DMEM). The material was centrifuged again, the supernatant was discarded, and the pellet was resuspended in DMEM supplemented with 10% fetal bovine serum (FBS) and a combination of penicillin (10 U/mL) and streptomycin (10 µg/mL). The material was transferred to 75 cm² culture flasks and stored in a 5% CO₂ incubator at 37°C until use. Vero cells (approximately 3x10⁵ cells/mL) were washed with PBS, transferred to microtiter plates, and inoculated with HSV-1 in a series of viral dilutions (between 10⁻³ and 10⁻⁷). Each viral suspension (HSV-1 strains) was inoculated into microtiter plates (100 µL per well), cultured in 10 mL of DMEM supplemented with 5% FBS and antibiotics/antifungal agents, and trypsinized 24 hours prior to viral infection. First, the DMEM culture medium was aspirated from the flask, and the cell monolayer was washed three times with phosphate-buffered saline (PBS). After this procedure, 100 µL of each viral dilution of HSV-1 was inoculated and incubated at 37°C in a 5% CO₂ incubator for 1 hour to allow viral adsorption and penetration.

A VERO cell line without HSV-1 inoculation was used as a control, aiding in determining toxic concentrations and exposure times for normal cells.

Cell viability test

The cytotoxic properties of Andiroba oil (Carapa guianensis) were previously evaluated on VERO E6 cells.

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To test cell viability, the Trypan blue dye exclusion method and the MTT colorimetric assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) were used. The tested AO concentrations (10 ng/mL and 100 ng/mL), when compared to the positive control, did not interfere with cell viability.

Evaluation of andiroba's antiviral action against HSV-1

The antiviral action of andiroba oil was evaluated by viral titration based on the reduction of viral cytopathic effect (CPE), an effective technique for assessing the antiviral action of a drug against HSV-1, using the observation and quantification of damage inhibition caused to cells by the virus (Yin et al., 2019).

In microtiter plates, immediately after the viral adsorption period (1 hour), Vero cells were washed again with PBS, and andiroba oil, dissolved in dimethyl sulfoxide (DMSO) at concentrations of 10 ng/mL and 100 ng/mL, was added to wells containing cells and HSV-1 in dilutions between 10^{-3} and 10^{-7} in triplicate, as described below:

- 1. Andiroba groups:** Cells with infection and AO use at concentrations of 10 ng/mL and 100 ng/mL.
- 2. Negative control:** Cells without infection and without AO use (cell control).
- 3. Positive control:** Cells infected with HSV-1 and without AO use (virus control).

After viral inoculation and treatment with andiroba, the plates were incubated in a CO₂ incubator at 37°C. Readings were made daily at 24h, 48h, and 72h to observe characteristic viral CPE (+) or not (-). Observations were performed using an inverted microscope, and CPE inhibition was compared to the controls. The cytopathic effect caused by HSV-1 includes changes characterized by cell rounding, disorganization of the monolayer, forming cell clusters or completely destroying the monolayer. To reduce subjectivity in the analysis, the observed images were captured and standardized (same magnification, lighting), and ImageJ software was used to count infected cells and determine the percentage of infected monolayers for subsequent CPE classification. CPE caused by HSV-1 was estimated in scores on a scale from 1 to 5 based on the degree of monolayer destruction (Fritz et al., 2007):

- 1. No CPE (0%):** The monolayer remains intact, with no signs of destruction or alteration.
- 2. Mild CPE (1 – 25%):** A small number of rounded or lysed cells, but most of the monolayer is still intact.
- 3. Moderate CPE (25 – 50%):** More significant cell rounding and lysis, but part of the monolayer is still partially preserved.
- 4. Extensive CPE (50 – 75%):** A large part of the monolayer is destroyed, with areas of rounded cells and many lysed cells, with significant detachment areas.
- 5. Total CPE (> 75%):** Almost complete or complete destruction of the monolayer, with few or no intact cells.

From the CPE values, the percentage of viral inhibition (% VI) was also calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{CPE_{test} - CPE_{cv}}{CPE_{cc} - CPE_{cv}} \times 100 \dots \dots \dots (1)$$

Where CPE test is the viral cytopathic effect of the treatment groups at different concentrations of andiroba oil, CPE cv is the viral cytopathic effect of the virus control, and CPE cc is the viral cytopathic effect of the cell control.

Statistical analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 24.0 (IBM). Descriptive statistics were applied, with absolute and relative frequencies for categorical variables and measures of central tendency and variability for continuous numerical variables. Results were calculated using original values and expressed as means. Statistical significance was assessed using the Student's t-test with Welch's correction and ANOVA. A p-value below 5% ($\alpha = 0.05$) was considered significant.

Results

Gas chromatography analysis (GC-MS)

Table 1 presents the gas chromatography analysis of 50% andiroba oil, detailing its composition and relative values. One hundred percent of the compounds were identified. Oleic acid was the most abundant component at 47.33%, followed by palmitic acid at 31.46%.

Evaluation of the antiviral action of andiroba against HSV-1

Based on the results obtained from prior cytotoxicity assays, the antiviral activity of andiroba was evaluated at non-cytotoxic concentrations against different viral dilutions. The concentrations analyzed were 10 ng/mL and 100 ng/mL of andiroba oil (AO) and HSV-1 dilutions ranging from 10^{-3} to 10^{-7} at intervals of 24, 48, and 72 hours. In this study, cell controls (Vero E6 cells without HSV-1 infection or treatment) and virus controls (Vero E6 cells infected with HSV-1 but without treatment) were used. The results were analyzed qualitatively and quantitatively using viral titration based on the reduction of cytopathic effect (CPE). The viral cytopathic effect of herpes simplex virus type 1 (HSV-1) in Vero cells is characterized by a series of typical morphological alterations caused by viral infection. These include cell rounding and detachment, cell fusion forming syncytia, loss of confluence, and the progressive fragmentation of the continuous cell layer (cell monolayer) as cells die or detach, leaving empty areas. Figure 1 illustrates the scoring system, ranging from 1 to 5, used to classify the CPE of HSV-1 in Vero E6 cells in this study. Table 2 presents the comparison of mean cytopathic effect (CPE) scores of HSV-1 in Vero E6 cells treated with andiroba oil (AO) at concentrations of 10 ng/mL and 100 ng/mL, compared to positive and negative controls across 24, 48, and 72 hours. When compared to the positive control (infected untreated cells), treatment with AO resulted in lower mean CPE values in most conditions. However, statistically significant differences were observed only in specific

comparisons. Notably, a significant reduction was observed for HSV-1 10⁻⁵ treated with AO at 100 ng/mL (mean = 2.33 ± 0.577) compared to the positive control (mean = 4.00 ± 1.00; *p* = 0.038). Other comparisons, such as HSV-1 10⁻³ and 10⁻⁷ at the same concentration, did not reach statistical significance (*p* = 0.057). At the concentration of 10 ng/mL, most comparisons with the

positive control did not show statistically significant differences (*p* > 0.05), although a consistent trend toward reduced CPE was observed. Comparisons with the negative control (uninfected cells) showed significantly higher CPE values in infected and treated groups, as expected, confirming the presence of viral-induced cytopathic damage even after treatment.

Table 1: Lipidic composition of andiroba oil -*Carapa guianensis*

Andiroba oil		
RT	Components	Composition (%)
7,199	Heptanoic acid	0,3989
13,674	Lauric acid	1,1572
15,883	Miristic acid	0,6394
17,982	Palmitic acid	31,4641
18,193	Palmitoleic acid	0,9184

18,862	Margaric acid	0,1154
19,845	Stearic acid	7,1279
20,064	Oleic acid	47,3356
20,428	Linoleic acid	8,9869
20,966	Linolenic acid	0,2501
21,530	Araquidic acid	1,0897
23,562	Behenic acid	0,5164

RT: Retention time

Table 2: Student's t-test for mean comparison at 24h, 48h, and 72h of HSV-1 CPE in Vero E6 cells treated with AO, based on concentrations compared to the control groups.

Variable	N	Mean	Median	Standard Deviation	Standard Error	Mean Difference	P-Value ⁽¹⁾
HSV-1 10 ⁻³ x AO 10 ng/mL	3	3.67	4.00	0.577	0.333		
Positive control – cell and HSV-1 10 ⁻³	3	4.00	4.00	1.000	0.577	-0.333	0.423 _{ns}
HSV-1 10 ⁻⁵ AO x 10 ng/mL	3	3.33	4.00	1.155	0.667		
Positive control – cell and HSV-1 10 ⁻⁵	3	4.00	4.00	1.000	0.577	-0.667	0.184 _{ns}
HSV-1 10 ⁻⁷ x AO 10 ng/mL	3	2.67	3.00	0.577	0.333		
Positive control – cell and HSV-1 10 ⁻⁷	3	3.67	4.00	0.577	0.333	-	-
HSV-1 10 ⁻³ x AO 10 ng/mL	3	3.67	4.00	0.577	0.333		
Negative control	3	1.00	1.00	0.000	0.000	2.667	0.015*
HSV-1 10 ⁻⁵ AO x 10 ng/mL	3	3.33	4.00	1.155	0.667		
Negative control	3	1.00	1.00	0.000	0.000	2.333	0.073 _{ns}
HSV-1 10 ⁻⁷ x AO 10 ng/mL	3	2.67	3.00	0.577	0.333		
Negative control	3	1.00	1.00	0.000	0.000	1.667	0.038*
HSV-1 10 ⁻³ x AO 100 ng/mL	3	2.67	3.00	0.577	0.333		
Positive control – cell and HSV-1 10 ⁻³	3	4.00	4.00	1.000	0.577	-1.333	0.057 _{ns}
HSV-1 10 ⁻⁵ x AO 100 ng/mL	3	2.33	2.00	0.577	0.333		
Positive control – cell and HSV-1 10 ⁻⁵	3	4.00	4.00	1.000	0.577	-1.667	0.038*
HSV-1 10 ⁻⁷ x AO 100 ng/mL	3	2.33	2.00	0.577	0.333		
Positive control – cell and HSV-1 10 ⁻⁷	3	3.67	4.00	0.577	0.333	-1.333	0.057 _{ns}
HSV-1 10 ⁻³ x AO 100 ng/mL	3	2.67	3.00	0.577	0.333		
Negative control	3	1.00	1.00	0.000	0.000	1.667	0.038*
HSV-1 10 ⁻⁵ x AO 100 ng/mL	3	2.33	2.00	0.577	0.333		
Negative control	3	1.00	1.00	0.000	0.000	1.333	0.057 _{ns}
HSV-1 10 ⁻⁷ x AO 100 ng/mL	3	2.33	2.00	0.577	0.333		
Negative control	3	1.00	1.00	0.000	0.000	1.333	0.057 _{ns}

Source: Own elaboration (2024). EPD = Standard error of the difference; DM = Mean difference; Note. H_a μ Measurement 1 - Measurement 2 ≠ 0, * Data are essentially constant.

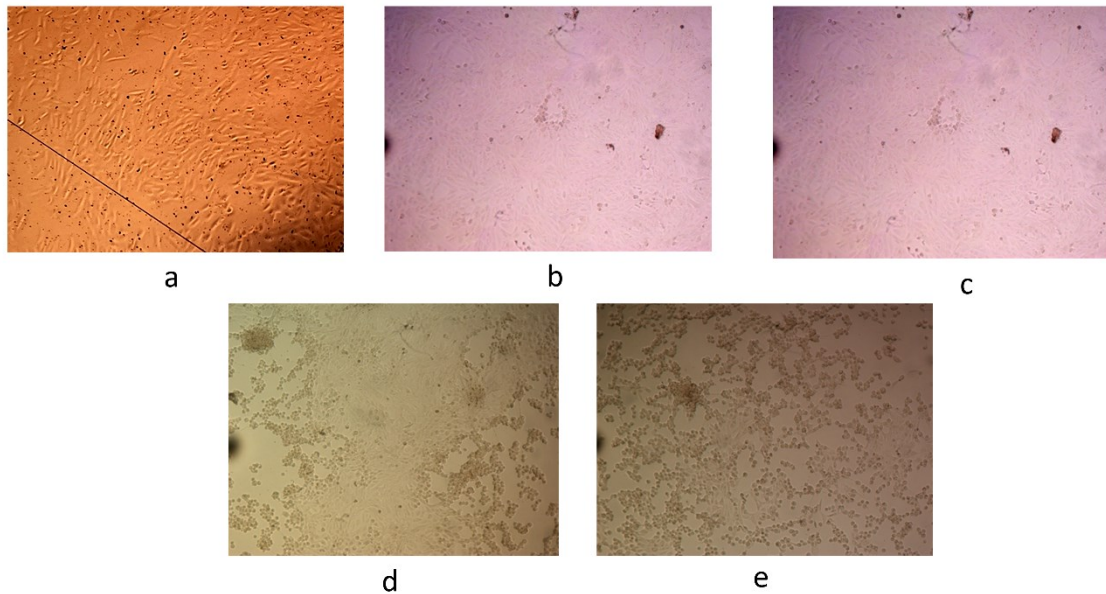
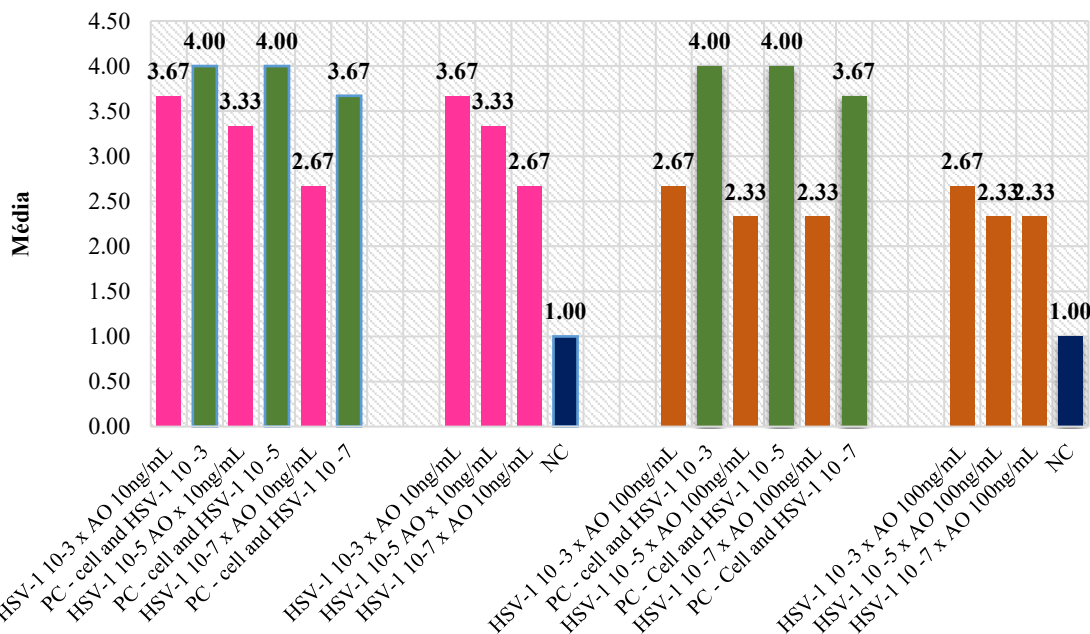


Figure 1: Images representing the CPE of HSV-1 in Vero E6 cells. a) Score 1 (No CPE): The cell monolayer remains intact, with a confluent Vero cell monolayer, showing no signs of destruction or alteration. b) Score 2 (Mild CPE): HSV-1 viral cytopathic effect between 1 and 25% of the infected Vero cell monolayer. A small number of rounded or lysed cells can be observed, but most of the cell monolayer remains intact. c) Score 3 (Moderate CPE): HSV-1 viral cytopathic effect between 25 and 50% of the infected Vero cell monolayer. More significant cell rounding and lysis are observed, but part of the cell monolayer remains partially preserved. d) Score 4 (Extensive CPE): HSV-1 viral cytopathic effect between 50 and 75% of the infected Vero cell monolayer. A large portion of the cell monolayer is destroyed, with areas of rounded cells and many lysed cells, showing significant detachment. e) Score 5 (Severe CPE): HSV-1 viral cytopathic effect with over 75% of the infected Vero cell monolayer. Almost complete or complete destruction of the cell monolayer, with few or no intact cells. (Photos by the author). (Image 10x)



Note: PC = Positive control; NC = Negative control.

Figure 2: Means of HSV-1 CPE in Vero E6 cells treated with AO, in relation to concentrations compared to the positive and negative control groups. The pink and orange bars represent treatments with andiroba oil at concentrations of 10 ng/mL and 100 ng/mL, respectively, compared to the positive control (green) and negative control (blue). It is observed that in all comparisons with the positive control, the CPE means with andiroba oil treatment were lower than the control means, except when compared to the negative control, where the treatment means were higher. However, it is noted that the means decrease according to the HSV-1 stratification: HSV-1 10⁻³ (3.67), HSV-1 10⁻⁵ (3.33), and HSV-1 10⁻⁷ (2.67) in the 10 ng/mL group, and HSV-1 10⁻³ (2.67), HSV-1 10⁻⁵ (2.33), and HSV-1 10⁻⁷ (2.33) in the 100 ng/mL group.

Table 3 shows that, at 10 ng/mL, the lowest mean CPE was observed at HSV-1 10⁻⁷ (mean = 2.67 ± 0.577), but no statistically significant differences were found among viral dilutions (*p* = 0.373). Similarly, Table 4 shows that at 100 ng/mL, the lowest CPE values were observed at HSV-1 10⁻⁵ and 10⁻⁷ (mean = 2.33 ± 0.577), with no significant differences between groups (*p* = 0.729). Although Figure 3 suggests increased viral inhibition at 100 ng/mL compared to 10 ng/mL, the absence of consistent statistical significance across comparisons indicates that a clear dose–response relationship cannot be conclusively established. Table 4 presents the comparison of mean

cytopathic effect (CPE) scores of HSV-1 in Vero E6 cells treated with andiroba oil (AO) at a concentration of 100 ng/mL across different viral dilutions. The lowest mean CPE values were observed for HSV-1 at dilutions of 10⁻⁵ and 10⁻⁷ (mean = 2.33 ± 0.58 for both conditions). These values correspond to a mild cytopathic effect, characterized by a limited number of rounded or lysed cells and relative preservation of the cell monolayer. Despite these numerical differences, no statistically significant differences were observed among the viral dilutions (*p* = 0.729), indicating that CPE reduction was not significantly influenced by viral dilution under this treatment condition.

Table 3: Student's t-test for mean comparison of HSV-1 CPE in Vero E6 cells after treatment with andiroba oil at a concentration of 10 ng/mL.

Factor	N (days)	Media	Standard deviation	CI de 95%	P-Value ⁽¹⁾
HSV-1 10 ⁻³ x AO 10 ng/mL	3	3,667	0,577	(2,513; 4,820)	0.373 _{ns}
HSV-1 10 ⁻⁵ x AO 10 ng/mL	3	3,333	1,155	(2,180; 4,487)	
HSV-1 10 ⁻⁷ x AO 10 ng/mL	3	2,667	0,577	(1,513; 3,820)	

Combined standard deviation = 0,816497

Table 4: Student's t-test for mean comparison of HSV-1 CPE in Vero E6 cells after treatment with andiroba oil at a concentration of 100 ng/mL.

Factor	N (days)	Media	Standard deviation	CI de 95%	P-Value ⁽¹⁾
HSV-1 10 ⁻³ x AO 100 ng/mL	3	2,667	0,577	(1,851; 3,482)	0.729 _{ns}
HSV-1 10 ⁻⁵ x AO 100 ng/mL	3	2,333	0,577	(1,518; 3,149)	
HSV-1 10 ⁻⁷ x AO 100 ng/mL	3	2,333	0,577	(1,518; 3,149)	

Combined standard deviation = 0,577350

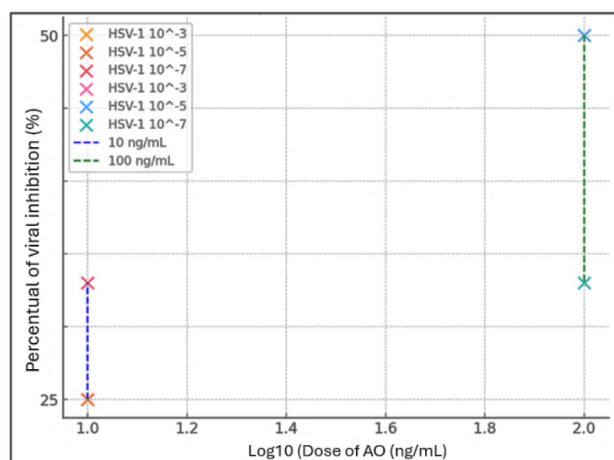


Figure 3: Dose-response curve of the antiviral activity of AO in Vero E6 cells infected with HSV-1 at concentrations of 10 ng/mL and 100 ng/mL of the oil. The graph shows the percentage of viral inhibition (y-axis) as a function of the logarithm of andiroba doses in ng/mL (x-axis). It can be observed that viral inhibition increases with a dose of 100 ng/mL compared to 10 ng/mL. The antiviral response appears to be more effective at higher viral dilutions when treated with the higher dose of andiroba (100 ng/mL).

Discussion

The search for new therapeutic alternatives against viral infections, such as Herpes Simplex Virus type 1 (HSV-1), has stimulated increasing interest in natural compounds with antiviral potential (Hussein; El-Anssary, 2018). In this

context, the biodiversity of the Amazon region represents an important source of bioactive substances, including those derived from medicinal plants traditionally used in folk medicine. Among these, andiroba oil (*Carapa guianensis*) has been widely reported for its anti-inflammatory, analgesic, and healing properties (Silva, 2018; Ribeiro et al., 2021; Amorim et al., 2022). However, studies specifically evaluating its antiviral activity against HSV-1 remain scarce. In the present study, treatment with andiroba oil was associated with a reduction in HSV-1-induced cytopathic effects (CPE) in Vero E6 cells, particularly at the concentration of 100 ng/mL. When compared to the positive control, treated groups consistently showed lower mean CPE values, suggesting a potential inhibitory effect on virus-induced cellular damage. Nevertheless, statistically significant differences were observed only in specific conditions, such as at the viral dilution of 10⁻⁵, indicating that the observed antiviral effect is modest and not consistent across all tested conditions. Although the graphical analysis suggests a greater reduction in CPE at higher concentrations of the oil, the statistical results do not robustly support a clear dose–response relationship. Therefore, the interpretation of a dose-dependent effect should be approached with caution, as the observed differences may reflect variability inherent to the experimental model and the limited sample size. The chromatographic analysis performed in this study demonstrated that andiroba oil is predominantly composed of fatty acids, particularly oleic acid (47.33%) and palmitic acid (31.46), consistent with previous reports (Silva, 2018; Milhomem-Paixão et al., 2016). These fatty acids have

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been associated with anti-inflammatory and immunomodulatory properties, which may contribute indirectly to antiviral activity (Souza et al., 2022). One possible mechanism involves alterations in membrane structure or interference with virus–host cell interactions, potentially affecting viral entry or replication. However, it is important to emphasize that no mechanistic assays were performed in this study, and such interpretations remain hypothetical. Medicinal plants are known to contain a wide range of secondary metabolites, including flavonoids, terpenoids, alkaloids, and phenolic compounds, many of which have demonstrated antiviral activity through different mechanisms, such as inhibition of viral replication, interference with viral entry, or modulation of the host immune response (Hussein; El-Anssary, 2018; Pavi et al., 2023; Pennisi et al., 2023). Although the phytochemical profile of andiroba oil was not directly evaluated in the present study, previous investigations have reported the presence of compounds such as phenols, tannins, saponins, steroids, and triterpenoids in *Carapa guianensis* (Amorim et al., 2022; Kikuchi et al., 2020). Therefore, any association between these compounds and the antiviral effects observed here should be considered indirect. In addition, Silva (2018) highlighted the presence of limonoids in andiroba oil, representing approximately 2% to 5% of its composition. These compounds have been associated with biological activities, including anti-inflammatory and immunomodulatory effects, which may also contribute to the biological properties of the oil. Similarly, Reis (2007) demonstrated that compounds isolated from *C. guianensis* were capable of modulating cytokine production (TNF- α and IL-10) in human monocytes infected with DENV-2, although direct antiviral activity was not demonstrated in that study. The antiviral activity in the present study was assessed using the reduction of cytopathic effects, a commonly employed method based on the visual evaluation of virus-induced cellular damage (Yin et al., 2019; Watanabe et al., 2021). Although this approach provides a practical and direct assessment, it is inherently semi-quantitative and subject to observer variability. In an attempt to reduce this limitation, ImageJ software was used to support the analysis, allowing a more standardized and objective evaluation of CPE. Even so, this method does not directly quantify viral replication. More robust methodologies, such as plaque reduction assays and quantitative PCR, are considered gold standards for antiviral evaluation, as they allow precise measurement of viral load and infectivity (Lopez-Alvarez et al., 2016). The absence of these techniques in the present study represents an important limitation, as it was not possible to determine pharmacological parameters such as IC₅₀ or to confirm antiviral activity at the molecular level. Additional limitations include the small sample size (n = 3), which reduces statistical power, and the inability to distinguish whether the observed effects are due to direct antiviral activity or indirect cytoprotective effects on host cells. Overall, the findings of this study suggest that andiroba oil may exert a modest inhibitory effect on HSV-1-induced cytopathic damage in vitro. However, further studies employing quantitative and mechanistic approaches are

necessary to confirm these findings and to better understand the potential role of *Carapa guianensis* as a source of antiviral compounds.

Conclusion

In summary, andiroba oil demonstrated a reduction in HSV-1-induced cytopathic effects in Vero E6 cells, particularly at the concentration of 100 ng/mL under specific experimental conditions. However, the observed effects were not consistently supported by statistical significance across all comparisons, and no clear dose–response relationship could be established. The antiviral activity observed may be associated with the presence of fatty acids and other compounds identified by GC-MS, although no direct mechanism of action was demonstrated. The study has important limitations, including the small sample size, the reliance on CPE-based assessment, and the absence of quantitative molecular assays and pharmacological parameter determination. Future studies should incorporate methods such as viral load quantification, plaque reduction assays, cytotoxicity evaluation, and compound isolation to better characterize the antiviral potential and mechanisms of action of andiroba oil against HSV-1.

Conflict of interests

The authors declare no conflicts of interest.

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