



Biomonitored phytochemical study for in vitro anti-arboviral activity of *Tecoma castaneifolia* (D. Don) Melch. (Bignoniaceae)

Adriana C. C. Reis (PG)^{1*}, Geraldo Célio Brandão (PQ)¹

¹ Escola de Farmácia, Universidade Federal de Ouro Preto (adriana.reis@ufop.edu.br)

RESUMO

Tecoma castaneifolia (Bignoniaceae) foi investigada por um estudo fitoquímico bio guiado para atividade antiviral *in vitro* frente aos arbovírus CHIKV, MAYV e ZIKV. O extrato etanólico dos caules e sua fração diclorometano (TCS-DF) apresentaram atividade antiviral com melhores índices de seletividade para TCS-DF. O fracionamento de TCS-DF levou ao isolamento do composto TC-01, parcialmente caracterizado como um derivado do ácido *p*-hidroxibenzóico, que demonstrou potente e seletiva atividade antiviral. Os resultados indicam que *T. castaneifolia* é uma promissora fonte de compostos bioativos e TC-01 possui potencial para o desenvolvimento de antivirais contra arbovírus (re)emergentes.

Palavras-chave: Zika virus, Mayaro virus, Chikungunya virus, terpene, antiviral.

Introduction

Arboviruses (arthropod-borne viruses) are transmitted primarily through the bites of infected mosquitoes, particularly those of the *Aedes* genus. These viruses are widely distributed and have significant global health and economic impacts (1). Among the arboviruses of major public health concern are *Alphavirus chikungunya* (CHIKV), *Alphavirus mayaro* (MAYV), and *Orthoflavivirus zikaense* (ZIKV). Currently, no specific antiviral treatments are available for these infections (2).

Natural products, especially plant-derived compounds, have played a critical role in drug discovery. Species from the Bignoniaceae family—particularly those in the *Tecoma* genus—are widely used in traditional medicine and are known for various pharmacological properties, including antiviral activity. Recently, our research group demonstrated the anti-ZIKV potential of extracts from selected *Tecoma* species (3).

In this context, the present study aims to carry out bio-guided fractionation of *Tecoma castaneifolia* (D. Don) Melch stems and to evaluate their antiviral activity against CHIKV, MAYV, and ZIKV.

Experimental

Extraction preparation and fractionation of *T. castaneifolia* stems

This study involving *Tecoma castaneifolia* (D. Don) Melch was registered in the SISGEN system under the code A64E37C. The stems of *T. castaneifolia* were extracted by percolation using 92.8% ethanol at room temperature. The resulting ethanolic extract (EE-TCS; 13.7 g) was subjected to liquid-liquid partitioning with solvents of increasing polarity: dichloromethane, ethyl acetate, and methanol:water (6:4 v/v). This procedure yielded the following fractions: dichloromethane fraction (TCS-DF, 5.0 g), ethyl acetate fraction (TCS-EF, 2.1 g), and aqueous fraction (TCS-AF, 5.5 g).

The TCS-DF fraction (4.9 g) was further subjected to silica gel column chromatography using an elutropic gradient of increasing polarity as elution solvents: *n*-hexane, *n*-hexane/chloroform (9:1), *n*-hexane/chloroform (1:1), chloroform, chloroform/ethyl acetate (9:1), chloroform/ethyl acetate (1:1), ethyl acetate, ethyl acetate/methanol (1:1), and methanol. Fraction 20, isolated as a white amorphous solid, was purified by washing with ethyl acetate, affording compound TC-

01 (120.6 mg). TC-01 was characterized by nuclear magnetic resonance (NMR) spectroscopy.

Cytotoxicity assay

Cytotoxicity was evaluated using 96-well plates and Vero cells through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell viability was assessed 72 hours post-treatment, and the results were expressed as the median cytotoxic concentration (CC₅₀). All experiments were performed in triplicate.

Antiviral assay

Antiviral activity against CHIKV, MAYV, and ZIKV was assessed in Vero cells using the MTT method in 96-well plates. Results were expressed as the median effective concentration (EC₅₀) measured 72 hours after infection. All assays were performed in triplicate. The Selectivity Index (SI) was calculated as the ratio CC₅₀/EC₅₀.

Inhibitory cytopathic effect (CPE) assay

To confirm the antiviral activity observed in the MTT assays, an inhibitory CPE assay was conducted in 6-well plates using Vero cells infected with CHIKV, ZIKV, or MAYV at a multiplicity of infection of 1. Infected monolayers were examined and photographed by optical microscopy 48 hours post-infection.

Results e Discussion

Bioguided phytochemical study of *T. castaneifolia* stems

The ethanolic extract from *T. castaneifolia* stems and its corresponding fractions: TCS-DF, TCS-EF, and TCS-AF were evaluated *in vitro* for cytotoxicity and antiviral activity against the arboviruses CHIKV, MAYV, and ZIKV (Table 1).

Table 1. Cytotoxicity and anti-arboviral activity against CHIKV, MAYV, and ZIKV in vitro evaluation.

	Vero CC ₅₀ (µg/mL)	CHIKV EC ₅₀ (µg/mL)	MAYV EC ₅₀ (µg/mL)	ZIKV EC ₅₀ (µg/mL)
EE-TCS	122.3 ± 1.2	48.98 ± 2.5	42.21 ± 2.7	94.23 ± 3.2
TCS-DF	189.2 ± 1.3	49.89 ± 3.9	48.62 ± 1.9	49.49 ± 4.6
TCS-EF	> 400	NA	51.19 ± 1.5	NA
TCS-AF	> 400	NA	NA	NA

NA: Not Active.



The stem ethanolic extract (EE-TCS) and the dichloromethane fraction (TCS-DF) exhibited antiviral activity against all three arboviruses, with SI values ranging from 2.9 to 1.3 for EE-TCS and from 3.8 to 3.9 for TCS-DF. The fractionation process enhanced the anti-arboviral activity, resulting in improved SI values.

Isolation and chemical characterization of TC-01

The TCS-DF fraction was selected for further phytochemical investigation using column chromatography, which yielded 53 subfractions. Subfraction 20 afforded a white amorphous solid (120.6 mg), designated as TC-01, which was partially characterized by NMR analysis. The antiviral activity of TC-01 was subsequently evaluated.

The one- and two-dimensional NMR analysis indicates that TC-01 is an ester derived from *p*-hydroxybenzoic acid with a long linear aliphatic chain.

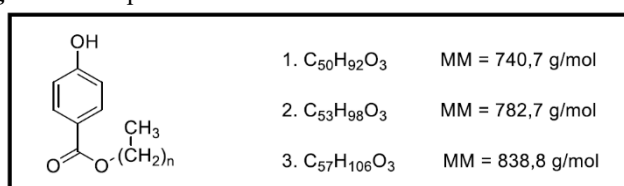
In the ^1H NMR spectrum, two doublets are observed at δ 6.72 and 7.05 ppm ($J = 8.5$ Hz; 2H each), consistent with a para-substituted aromatic system. The signal at δ 4.20 ppm (triplet, $J = 7.1$ Hz) was assigned to a CH_2 group bonded to oxygen (present in the ester linkage), while the signals at δ 2.83 and 2.23 ppm correspond to adjacent CH_2 groups. Multiple signals between δ 1.56–0.85 ppm represent the methylene groups and the terminal methyl group of the long aliphatic chain.

In the ^{13}C NMR spectrum, the signal at δ 174.18 ppm confirms the presence of a carbonyl group ($\text{C}=\text{O}$) typical of esters. Signals at δ 115.52 and 130.25 ppm, with higher intensity, confirm the symmetry of the para-substituted aromatic ring. The signal at δ 154.47 ppm indicates an aromatic carbon bonded to oxygen, characteristic of a phenolic hydroxyl group.

The HSQC spectrum confirms the ^1H – ^{13}C correlations for the aromatic and aliphatic chain groups. The HMBC shows correlations between the oxygenated CH_2 group and the carbonyl carbon, as well as between the aromatic hydrogens and the oxygenated carbon, supporting the proposed structure.

To determine the size of the aliphatic chain the exact mass of the compound is necessary. In Figure 1, are proposed possible chemical structures for the derivative of the *p*-hydroxybenzoic acid – TC-01.

Figure 1 – Proposed chemical structures for TC-01



Anti-arboviral evaluation of TC-01

The results of the biological assays of TC-01 are summarized in Table 2.

Table 2. Cytotoxicity and anti-arboviral activity against CHIKV, MAYV, and ZIKV in vitro evaluation.

	Vero CC ₅₀ ($\mu\text{g/mL}$)	CHIKV EC ₅₀ ($\mu\text{g/mL}$)	MAYV EC ₅₀ ($\mu\text{g/mL}$)	ZIKV EC ₅₀ ($\mu\text{g/mL}$)
TC-01	44.55 $\pm$ 1.5	10.51 $\pm$ 1.9	6.16 $\pm$ 1.5	9.31 $\pm$ 1.7
Ribavirin	370.40 $\pm$ 1.2	NT	105.30 $\pm$ 2.2	105.80 $\pm$ 1.6
Amantadine	84.89 $\pm$ 1.2	63.37 $\pm$ 1.7	NT	NT

NT: Not Tested.

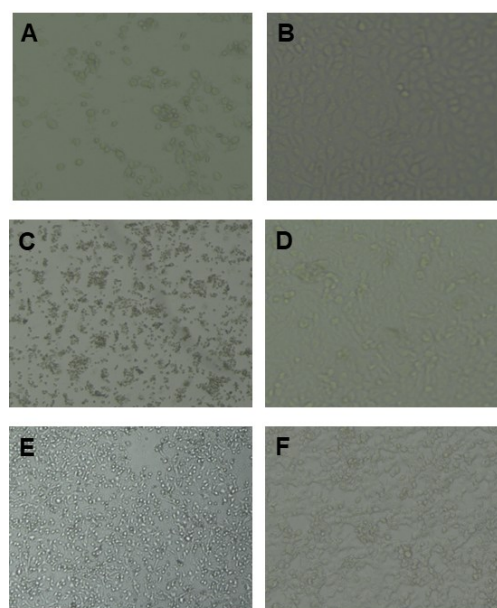
The isolated compound TC-01 inhibited the replication cycle of the arboviruses ZIKV, CHIKV, and MAYV, with EC₅₀ values ranging from 6.16 to 10.51 $\mu\text{g/mL}$ and SI between 4.3 and 7.4.

Compared to the crude extract and the dichloromethane fraction, TC01 was at least 4.7 times more active against the tested arboviruses. When compared to positive controls, it was 11.4 times more active against ZIKV than ribavirin, 6.0 times more active against CHIKV than amantadine, and 16.8 times more active than ribavirin in inhibiting MAYV replication. These in vitro antiviral results against the three arboviruses are unprecedented.

Inhibitory CPE of TC-01

To confirm the antiviral activity of TC-01 observed in the MTT colorimetric assay, a cytopathic effect (CPE) inhibition assay was conducted against the three arboviruses, and the cell monolayers were photographed 48 hours post-infection, as shown in Figure 2.

Figure 2 – Inhibitory CPE of TC-01 against CHIKV, MAYV, and ZIKV.



A: CHIKV viral control, **B:** TC-01 (10 $\mu\text{g/mL}$) anti-CHIKV activity, **C:** MAYV viral control, **D:** TC-01 anti-MAYV activity (10 $\mu\text{g/mL}$), **E:** ZIKV viral control, **F:** TC-01 anti-ZIKV activity (10 $\mu\text{g/mL}$).

Conclusion

Tecoma castaneifolia has proven to be a valuable source of bioactive compounds. The *p*-hydroxybenzoic acid derivative TC-01, isolated from the dichloromethane fraction of its stem extract, demonstrated potent and selective antiviral activity against ZIKV, CHIKV, and MAYV. TC-01 effectively inhibited the replication cycle of these arboviruses, highlighting its potential as a promising lead compound for the development of novel antiviral agents targeting (re)emerging arboviral infections.

Acknowledgments

We would like to thank FAPEMIG, CAPES, CNPq, UFOP.

References

1. A. Lim et al., Nat. Commun. **2025**, 23, 16, 1-13.
2. S.C. Weaver et al., Annu. Rev. **2018**, 69, 395-408.
3. B. Mahmoud et al., J. Adv. Biomed. Pharm. Sci. **2019**, 2,83-97.