BRAZILIAN RED PROPOLIS CONSTITUENTS AGAINST CHIKUNGUNYA VIRUS: *IN VITRO* AND *IN SILICO* INSIGHTS

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ABSTRACT

Introduction: Arboviruses are infections caused by viruses transmitted via arthropod vectors and are typically characterized by acute febrile illness often accompanied by exantematous rashes. Among the various arboviruses, those transmitted by mosquitoes of the genus *Aedes sp*. are particularly significant, especially infections caused by the Chikungunya virus (CHIKV). In recent years, outbreaks of this arbovirus have resulted in persistent polyarthralgia, which can last for months or even years. Currently, there are no licensed antiviral treatments available for this virus. **Objectives:** In this context, the present study evaluated the effects of 12 Brazilian red propolis (BRP) constituents (biochanin A, p-coumaric acid, pinocembrin, catechin hydrate, formononetin, coumarin, caffeic acid, isoliquiritigenin, ferulic acid, 2-coumaric acid, daidzein and chrysin) against CHIKV in Vero E6 cells *in vitro*. **Methods:** Initially, cytotoxicity assays and antiviral screenings were performed via the MTT cell viability assay with 12 BRP compounds individually at a concentration of 50 μM. Promising compounds identified in the antiviral screening were subjected to serial dilutions to determine the maximum nontoxic concentration (MNTC), CC_{50}, EC_{50}, and selectivity index (SI) values. For compounds with the highest SI, the percentage of CHIKV-positive cells was determined via intracellular flow cytometry, and cell supernatants were collected to quantify the absolute number of viral RNA copies via RT–qPCR. Additionally, a time-of-addition assay was conducted to evaluate antiviral activity at different times before or after infection. *In silico* analysis was performed to identify potential interactions between these compounds and CHIKV targets through *molecular docking.* **Results and discussion:** Of the 12 compounds evaluated, 6 demonstrated antiviral activity in screening assays (biochanin A, pinocembrin, catechin hydrate, formononetin, isoliquiritigenin and chrysin). Among these compounds, isoliquiritigenin (SI = 4.87) and catechin hydrate (SI = 18.31) were notable for their high selectivity indices and significant reductions in the percentage of infected cells. In the quantification of viral RNA copy numbers, the untreated infected control group (CHIKV) had 3.2×10^6 viral RNA copies, while isoliquiritigenin reduced this number to 9.4×10^4 copies, and catechin hydrate reduced it to 4×10^4 viral RNA copies. In the time-of-addition assay, the compounds did not exhibit significant antiviral activity at -2 h or during viral adsorption (0 h). However, both demonstrated promising antiviral activity up to 8 hours postviral adsorption in the posttreatment assay. *In silico* analyses revealed strong interactions between these two compounds and the CHIKV nsP3 protein. These findings suggest that BRP compounds may inhibit the viral replication cycle of CHIKV. **Conclusion:** In summary, the constituents of Brazilian red propolis, isoliquiritigenin and catechin hydrate, demonstrate significant antiviral activity against CHIKV *in vitro,* paving the way for the future development of novel bioactive antiviral agents.

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