Green Propolis-loaded Lipid Nanostructures Reduce SARS-CoV-2 Replication and Inflammation

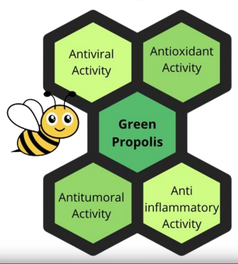
Iasmin R.S. Ferreira, Isabela A. Justino, Ronaldo Martins, Thais M. de Lima, Maria Vitória O. Souza, Jairo K. Bastos,
Priscyla D Marcato

School of Pharmaceutical Science of Ribeirão Preto, University of São Paulo

pmarcato@usp.br

INTRODUCTION

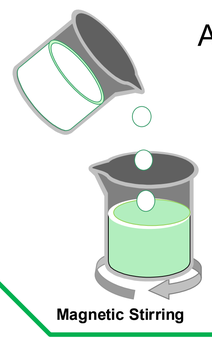
Green propolis, a resinous substance collected by bees from *Baccharis dracunculifolia* plants, is known for its potent biological properties, including antioxidant, anti-inflammatory, antiviral, and antitumor activities. However, this extract exhibits poor water solubility, instability under certain conditions, a strong taste, and low bioavailability. Therefore, this study aimed to encapsulate green propolis extract in lipid nanostructures and evaluate its biological effects against SARS-CoV-2.



METHODOLOGY

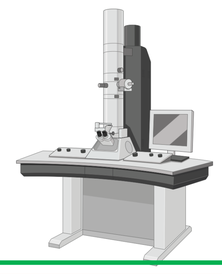
Preparation and characterization of ME-GP

Microemulsion preparation



Size evaluation, PdI, Zeta potential and particle concentration

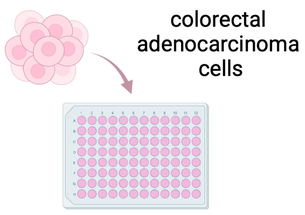
Morphology and size by TEM



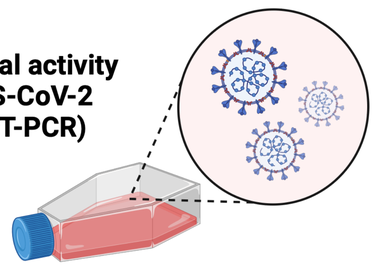
Antioxidant activity (DPPH assay)

Biological activity of ME-GP and free green propolis (GP)

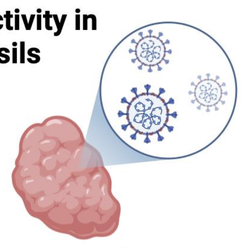
Cytotoxicity assay (Neutral Rede assay)



Antiviral activity SARS-CoV-2 (RT-PCR)

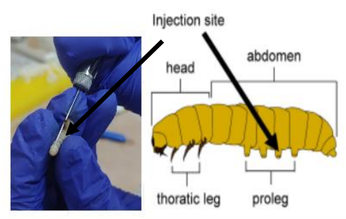


Antiviral activity in human tonsils



Committee on Ethics in Human number 4.465.408

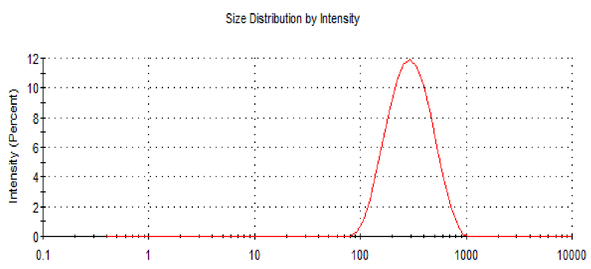
Toxicity assay in *Galleria mellonella* larvae



RESULTS

Microemulsion preparation and characterization

The microemulsion containing green propolis (ME-GP, 110 mg/mL) had a size of 217 ± 39.3 nm, a polydispersity index below 0.35, negative zeta potential (-24.3 ± 5.45 mV) and spherical morphology measured by TEM. ME-GP was found to be stable by DLS analysis over 180 days.



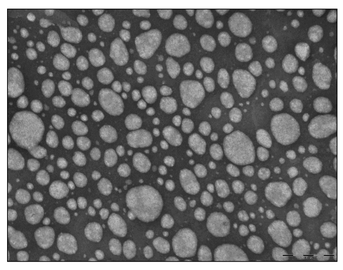


Figure 1. A) Size profile of ME-GP; B) TEM image of ME=GP

Antioxidant Activity

The determination of the antioxidant activity based on the reduction of the DPPH radical shows that the antioxidant activity of the ME-GP is greater than the antioxidant activity of the green propolis extract, with EC_{50} of 10 µg/mL and 29.1 µg/mL, respectively.

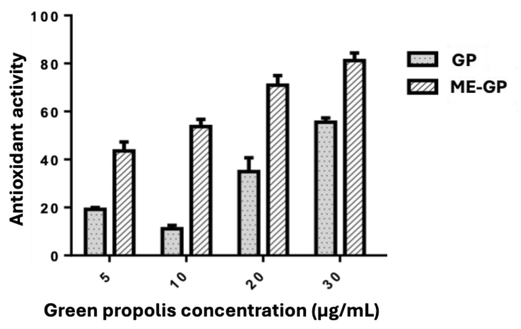


Figure 2. Antioxidant activity of green propolis (GP) and ME-GP

Cytotoxicity on Caco-2 cells

Nanoencapsulation also enhanced the antitumor activity of green propolis, with an IC50 2.5-fold lower (40.87 µg/mL) than pure extract (101.9 µg/mL) in colorectal adenocarcinoma cells (Caco-2).

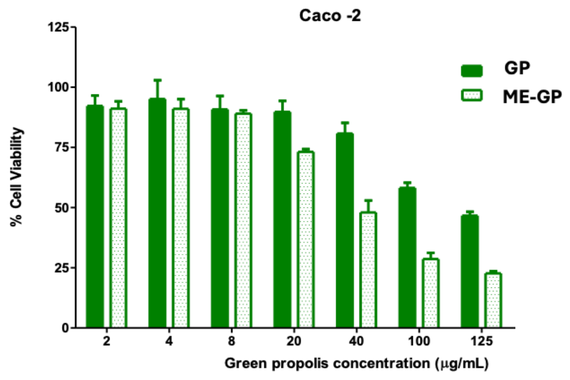


Figure 3. Cytotoxicity of green propolis (GP) and ME-GP on Caco-2 cells

Antiviral activity against SARS-CoV-2

The ME-GP demonstrated dose-dependent antiviral activity against SARS-CoV-2. At 15 µg/mL of propolis extract, for the WT and Delta variants, free-GP reduced the viral load by 70% and 30%, respectively, whereas ME-GP achieved reductions of 91.6% and 99% (Fig. 4).

Artepillin C, the major or most abundant compound in GP reduced the viral load by 67% and 87% at 15 and 30 µg/mL, showing relevant antiviral activity but lower than the ME-GP (Fig. 5)

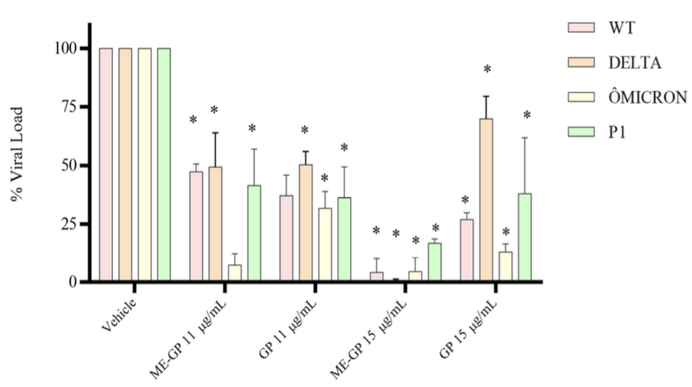


Figure 4. Virucidal activity of green propolis (GP) and ME-GP against SARS-CoV-2

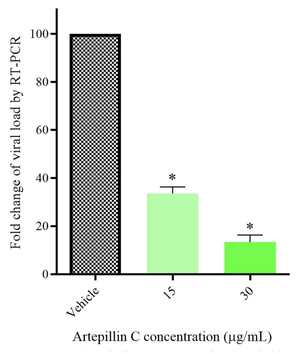


Figure 5. Antiviral activity of artepillin C against SARS-CoV-2 against SARS-CoV-2

Antiviral activity in human tonsils

- Viral load was reduced by over 80% in palatine tonsils infected with SARS-CoV-2 and treated with ME-GP (Fig 6A).
- ME-GP significantly decreased the levels of IL-6, IL-1β, and TNF-α compared with the negative control, indicating its potent anti-inflammatory activity (Fig. 6b)

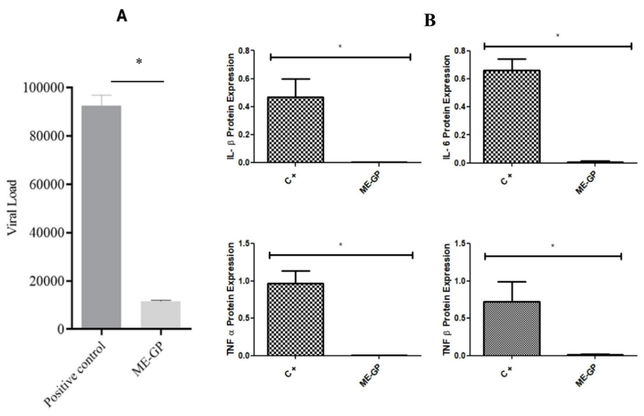


Figure 6. A) Antiviral activity in human tonsils treated with Brazilian green propolis encapsulated in microemulsion (ME-GP, 125 µg/mL) for 5 days, (B) Levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in human tonsils treated with ME-GP.

Toxicity assay in *Galleria mallonella* larvae

The survival curves in Galleria mellonella indicate that green propolis (GP) is well tolerated even at high doses (up to 2000 mg/kg), with survival rates above 80%. Although the nanoformulation (ME-GP) showed some toxicity at higher concentrations, these effects were observed only at doses far above those used by day, reinforcing its safety at therapeutically relevant levels.

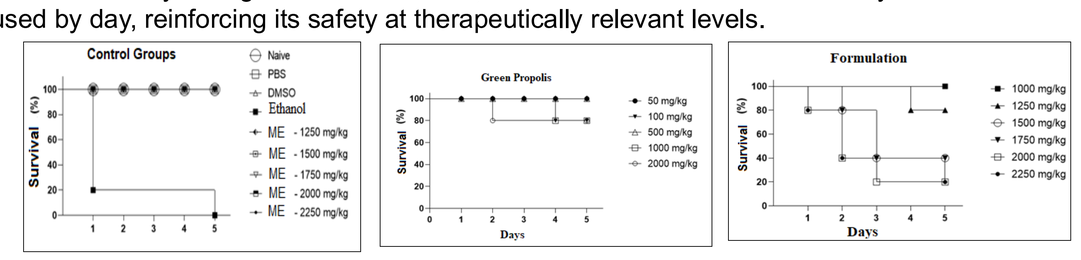


Figure 7. Survival of Galleria mellonella larvae after administration of control solutions, green propolis extract, and its nanoformulations at different doses over 5 days.

Financial Support

FAPESP
CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
CAPES
GRUPO APISVIDA

CONCLUSION

Taken together, the results indicate that ME-GP exhibits enhanced antiviral, anti-inflammatory, antioxidant, and antitumor activities compared to free GP, with favorable stability and safety profiles, supporting its potential as a multifunctional therapeutic formulation