

OP-039 [Aphitherapy]
Optimizing Bioactivity and Safety: Synergistic Effects of Green and Red Propolis Extracts for Enhanced Therapeutic Potential

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BACKGROUND: Brazilian propolis has been classified into 13 distinct types based on its chemical composition. Among them, green and red propolis are of particular importance due to their biological properties, production, and commercialization. Green propolis is derived from *Baccharis dracunculifolia*, while red propolis originates from *Dalbergia ecastophyllum* and *Symphonia globulifera*. Their chemical profiles differ significantly, with green propolis being more extensively studied and well-documented in terms of safety and clinical trials. Red propolis exhibits higher potency in some biological models; however, only three safety studies have been conducted to date, with one reporting an estrogenic effect in males.

PURPOSE: Given these differences, this study aimed to determine whether a 1:1 combination of green and red propolis could offer synergistic biological benefits while potentially reducing adverse effects. **METHODOLOGY:** Green, red, and combined propolis extracts were analyzed for total phenolic and flavonoid content (Folin-Denis and AlCl methods) and chemical profile (HPLC/DAD). Antioxidant capacity (DPPH, IC), antimicrobial activity (MIC, *Staphylococcus aureus*), anti-inflammatory effects (RAW264.7 cells), and cytotoxicity (MCF7, HeLa, AGS, Caco2, and HFF1 cells) were assessed. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test ($p < 0.05$). **RE**

SULTS: The chemical characterization of green, red, and mixed propolis extracts revealed total flavonoid contents of 65.20 ± 0.39 , 57.84 ± 0.20 , and 61.01 ± 0.36 mg/g (as quercetin), and total phenolic contents of 152.41 ± 1.18 , 160.95 ± 2.00 , and 162.89 ± 6.11 mg/g (as gallic acid), respectively. HPLC/DAD analysis identified key biomarkers: drupanin, artepellin C, and baccharin in green propolis, while formononetin, medicarpin, and vestitol were characteristic of red propolis, corroborating existing scientific data. Antioxidant capacity (IC) was determined as 11.28 ± 0.44 , 14.77 ± 0.40 , and 10.46 ± 0.34 $\mu\text{g/mL}$ for green, red, and mixed extracts, respectively. The MIC values against *S. aureus* were 1.003 ± 0.54 , 0.430 ± 0.0 , and 0.215 ± 0.00 mg/mL. In both anti-inflammatory and cytotoxicity assays, the combined extract consistently exhibited the most potent effects, suggesting a synergistic interaction.

CONCLUSION: These findings indicate that, in the models tested, combining green and red propolis may allow for lower effective concentrations while potentially mitigating the adverse effects associated with red propolis, if any.

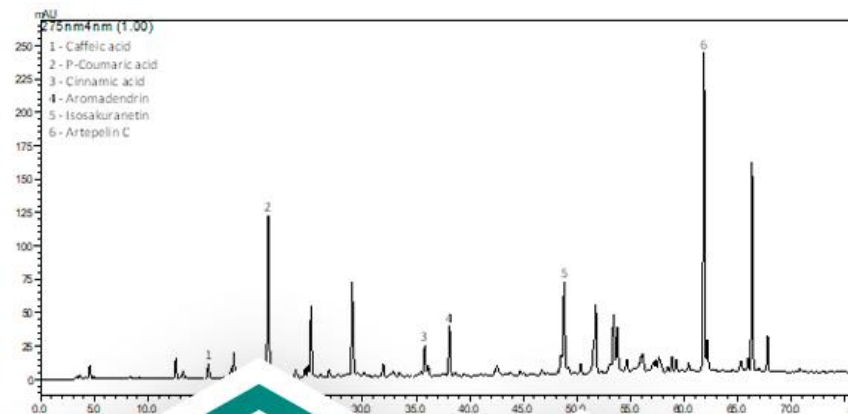


"Optimizing Bioactivity and Safety: Synergistic Effects of Green and Red Propolis Extracts for Enhanced Therapeutic Potential"

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Green Propolis



Green Propolis – Safety Aspects

- **General Safety**

- **Preclinical studies:** Non-toxic at therapeutic doses; LD₅₀ not reached in rodents.

- **Sub-chronic administration** (oral, 90 days): no major histopathological alterations.

(Sforcin et al., 2012; Paulino et al., 2008)

- **Human Clinical Trials**

- **COVID-19 adjunct trials, Health Volunteers, HIV-patients, Chronic kidney diseases patients**

- Doses up to **900 mg/day** well tolerated.

- Reported adverse events: rare, mild gastrointestinal discomfort or allergic reactions.

(Silveira et al., 2021, Diniz et al. 202~0; Silveira et al. 2019; Taska et al.; etc)

- **Cytotoxicity / Genotoxicity**

- **In vitro:** Non-genotoxic; antioxidant properties protect DNA from oxidative damage.

- Selective cytotoxicity against tumor cells, sparing normal cells.

(De Souza et al., 2019; Bueno-Silva et al., 2017)

Red Propolis Botanical Source

Produced by *Apis mellifera* bees.

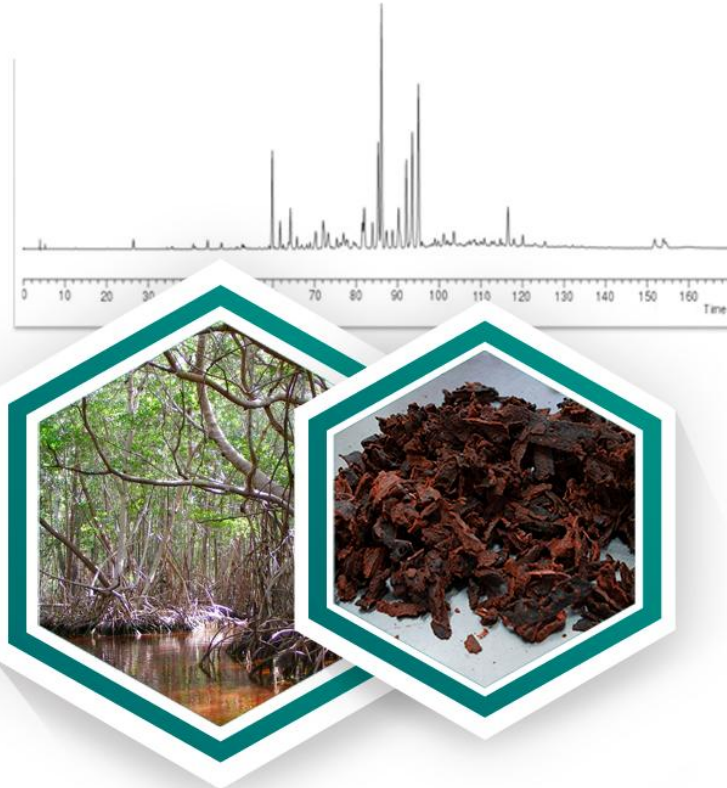
- Two sources (Ccana-Ccapatinta et al., 2020):

Primary - *Dalbergia ecastaphyllum* (L.) Taub.;

Secondary - *Symphonia globulifera* L.f.

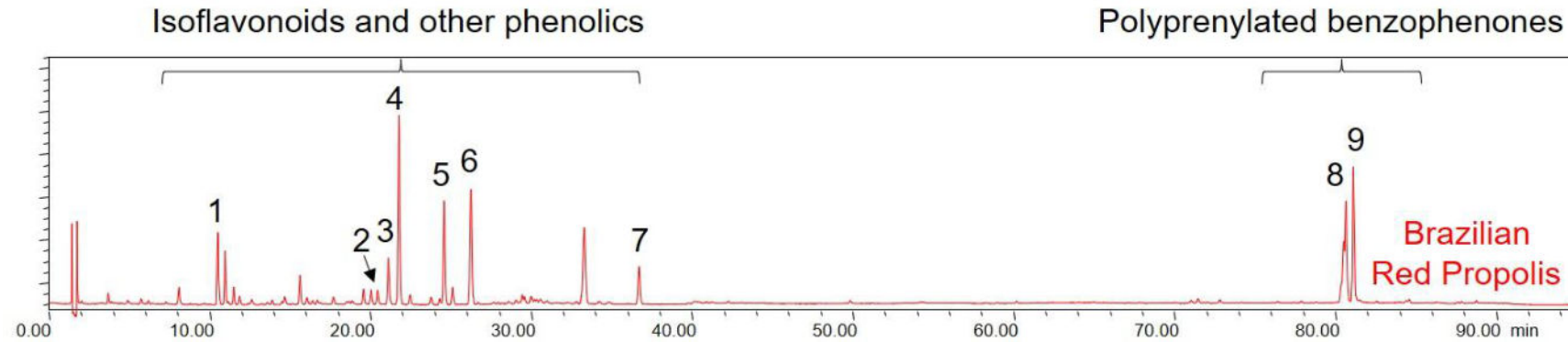


Red Propolis Botanical Source



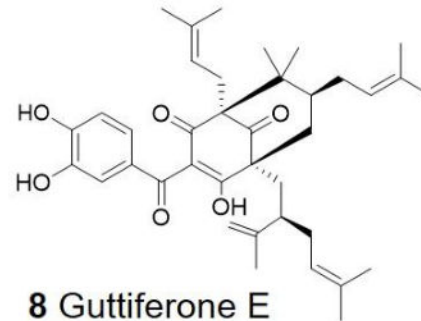
Dalbergia ecastaphyllum

Red Propolis

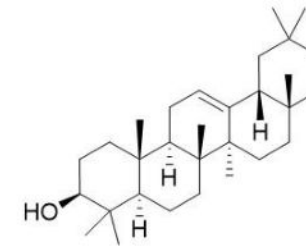


Symphonia globulifera L.f

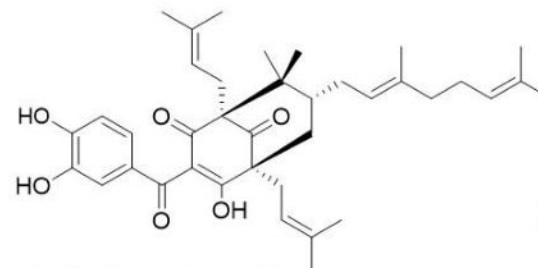
Botanical
Source →



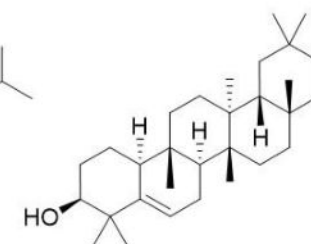
8 Guttiferone E



10 β -Amyrin



9 Oblongifolin B



11 Glutinol

Ccana-Ccapatinta et
al. 2020

Red Propolis – Safety Aspects

•General Safety

- Acute toxicity (rats): LD₅₀ > 300 mg/kg (oral); no deaths observed.
- Sub-chronic toxicity (90 days, up to 1000 mg/kg/day): some biochemical changes, **no histopathological organ damage**.
- Genotoxicity tests: **no DNA damage detected**.

(Journal of Ethnopharmacol. 2015; Food Chem Toxicol. 2022)

•Cytotoxicity

- At higher concentrations, **cytotoxic to keratinocytes and fibroblasts** — safe dose depends on extract type & concentration.

(BMC Complement Altern Med. 2015)

•Endocrine / Gender Effects

- Rich in **isoflavones** → possible **estrogen-like activity**.
- Some biochemical changes more pronounced in **male rats**.

(J Ethnopharmacol. 2015)

•Human Data

- No clinical safety studies** specific to red propolis.

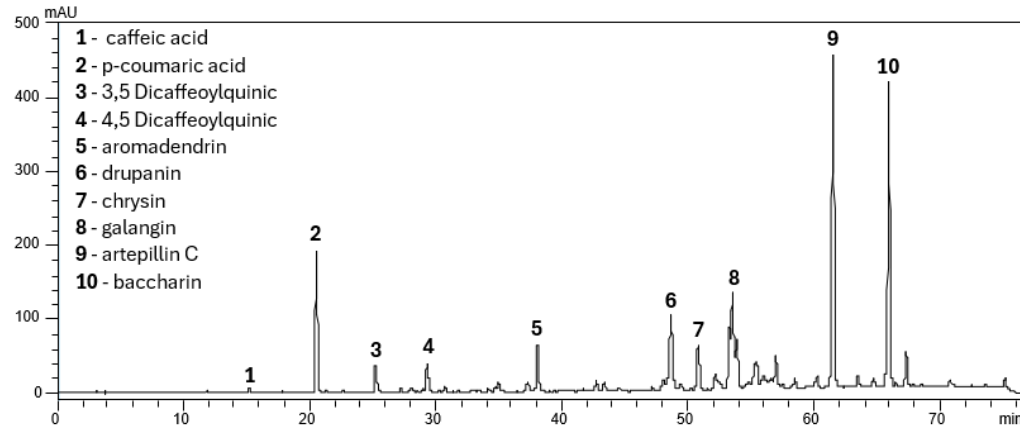
Aims of this Work

- Propose a synergic composition using green and red propolis
- Aiming to increase the effectiveness using lower dosages of each
- Determine the Chemical Characterization of each extract: green, red and the combination of green and red (1:1)
- Determine the Biological Effects

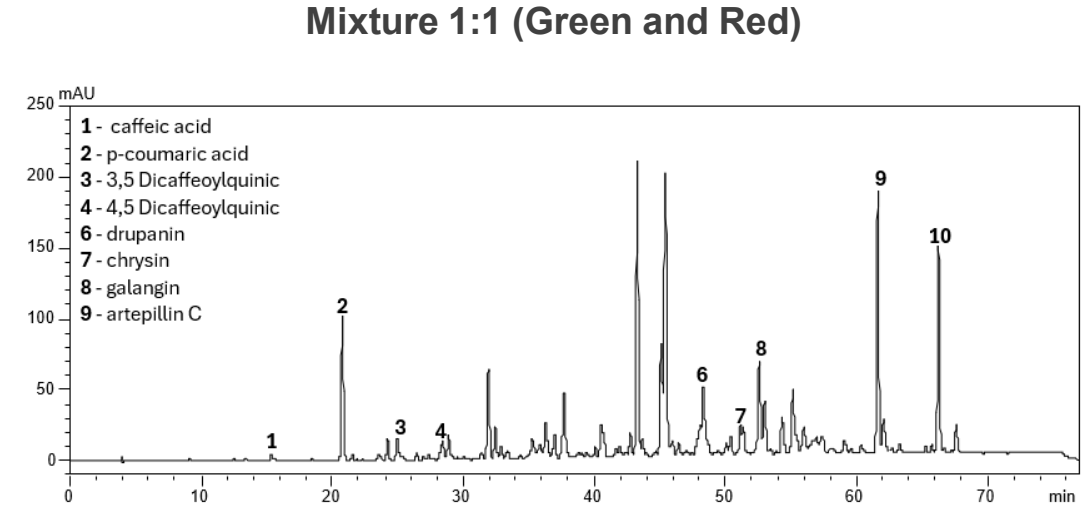


Chemical Characterization - Results

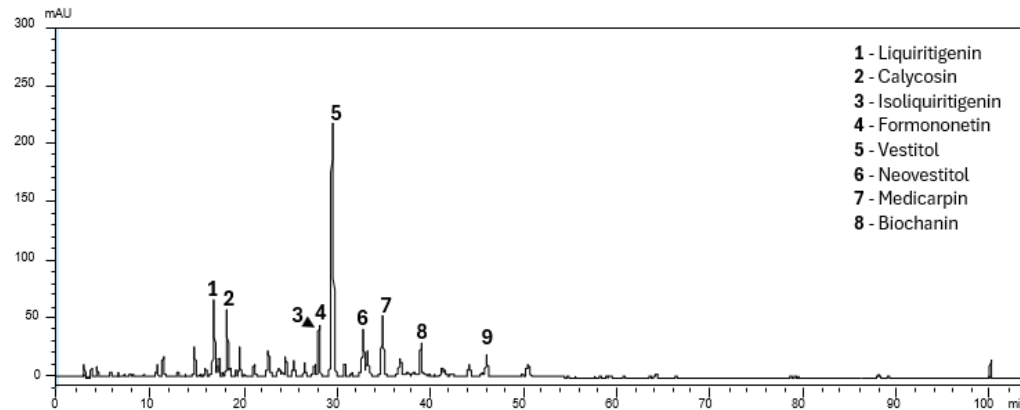
Chemical Characterization



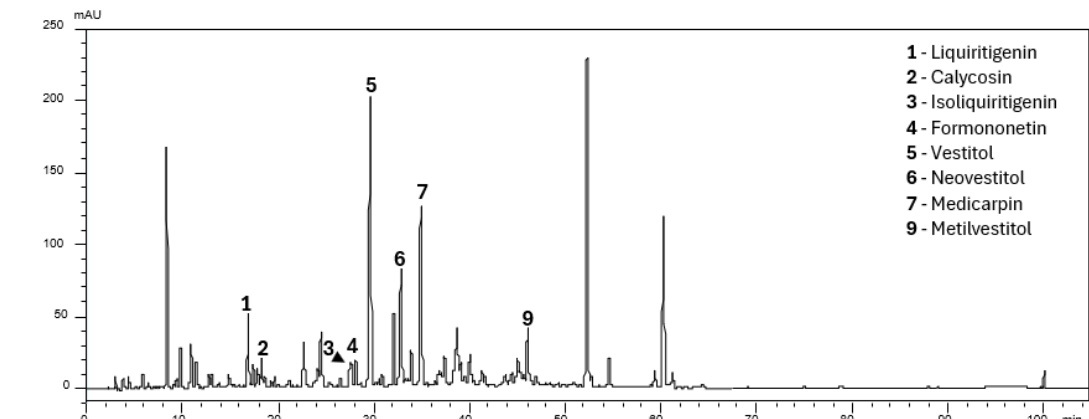
Green Standardized Propolis EPP-AF extract



HPLC Method for Green – ISO 24.381/2023

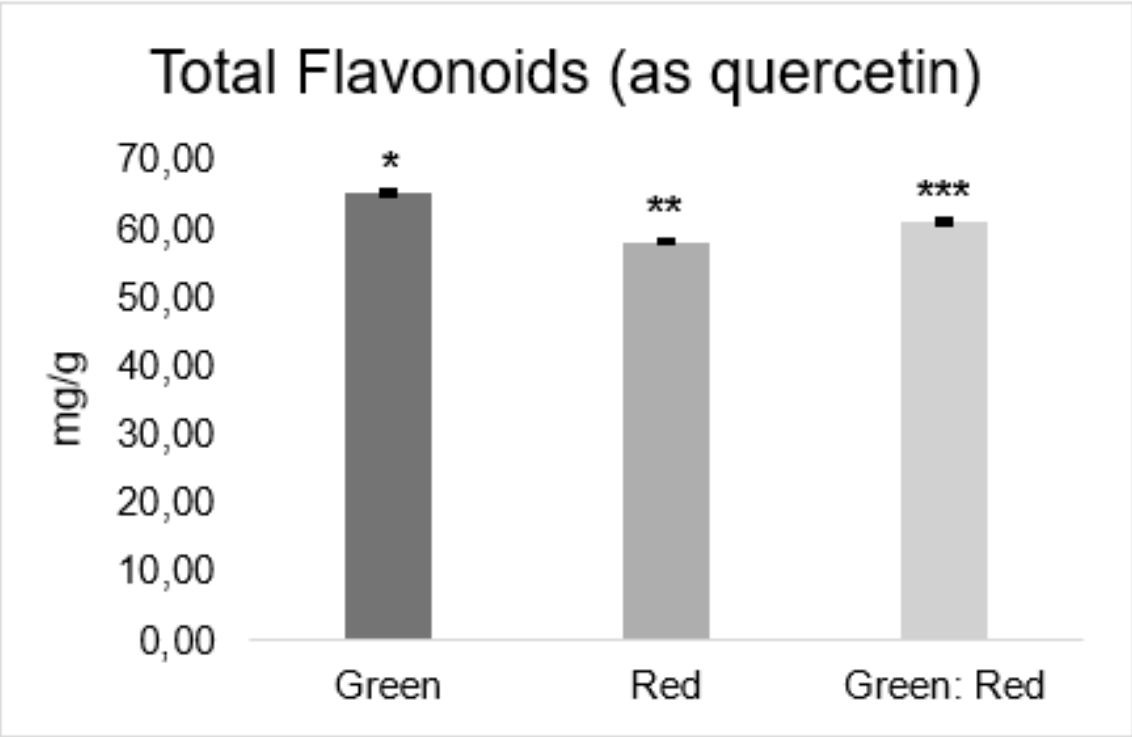


Red Standardized Propolis extract

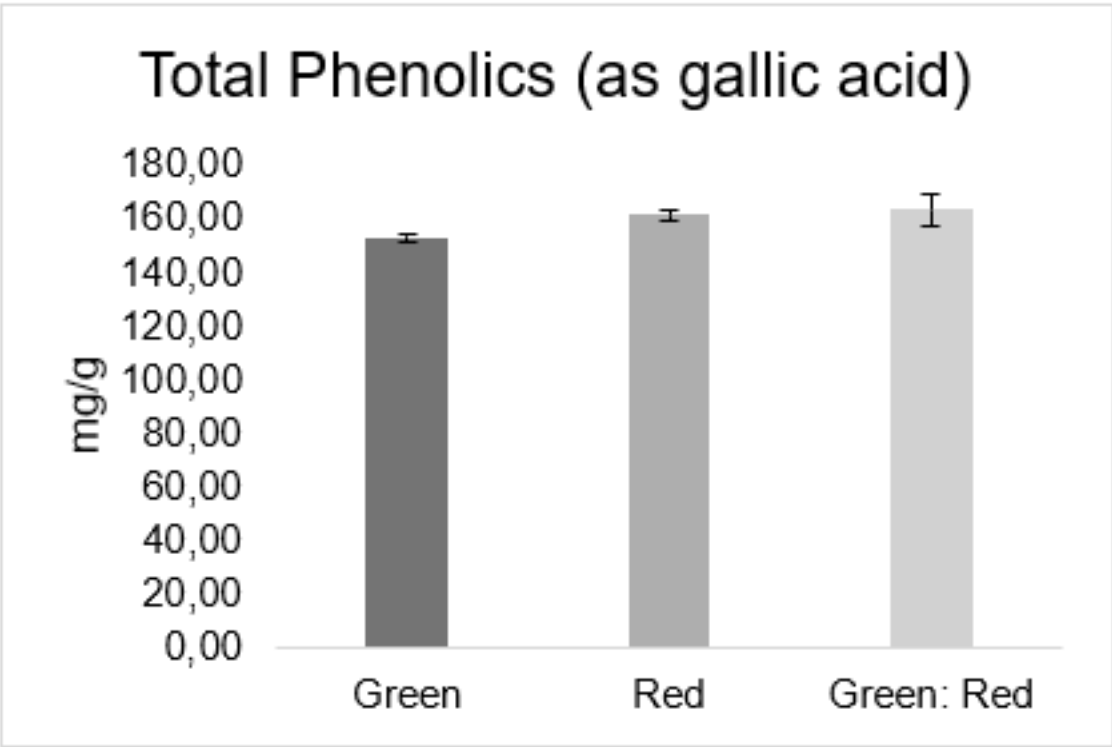


HPLC Method for Red – ISO 24.381/2023

Chemical Characterization



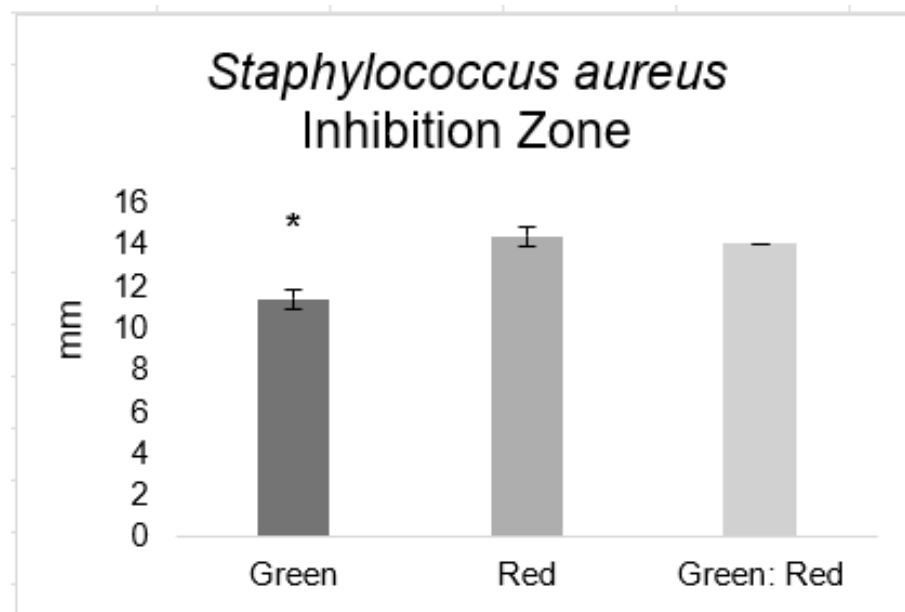
One Way ANOVA, with Tukey test (95% confidence)



Colorimetric Methods – ISO 24.381/2023

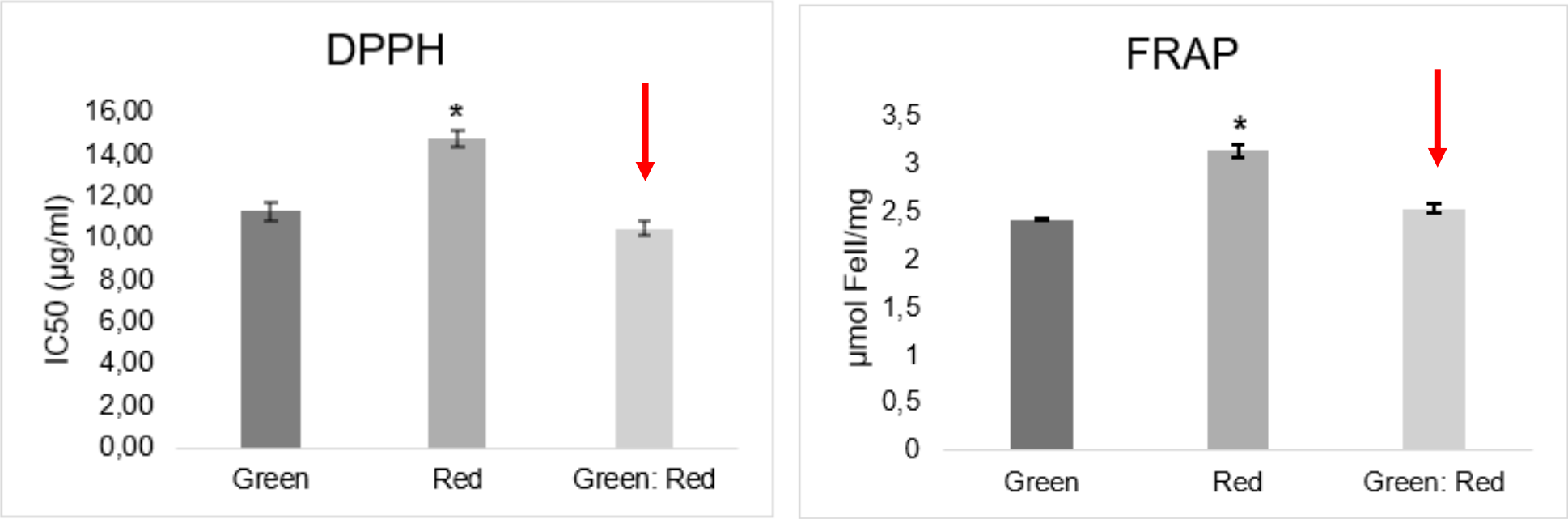
Biological Effects - Results

Antimicrobial and Antioxidant Effects



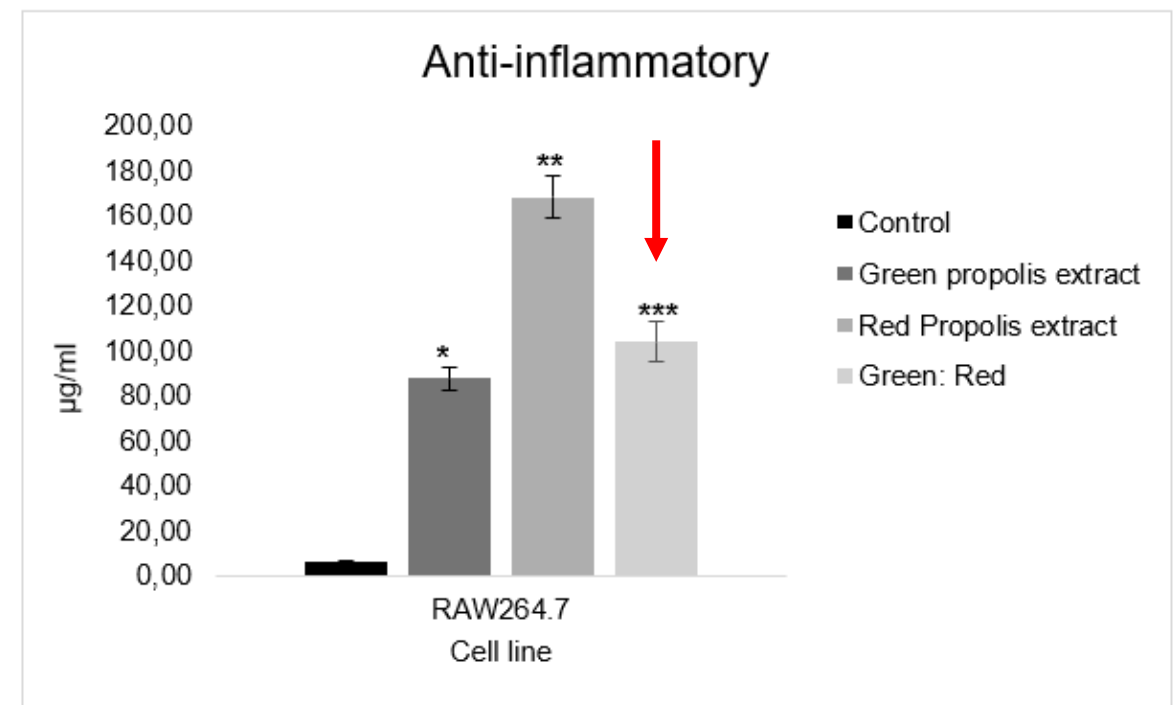
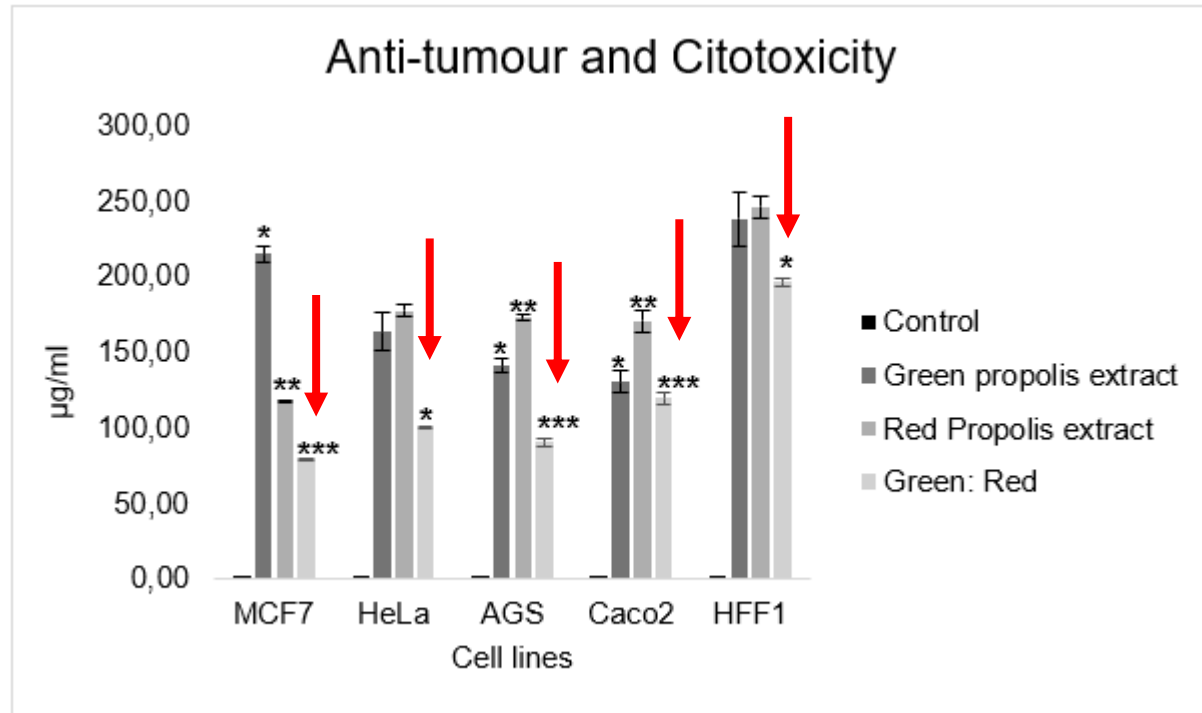
One Way ANOVA, with Tukey test (95% confidence)

Antioxidant Effects



One Way ANOVA, with Tukey test (95% confidence)

Anti-tumour, Cytotoxicity and Antii-nflammatory Effects



Conclusions

Conclusions

- The formulation was successfully obtained;
- The Synergy between green and red propolis was demonstrated for the following biological effects:
 - Antioxidant
 - Anti-tumoral
 - Anti-inflammatory
- It is possible to reduce the effective dosage with the combination, reducing the impact of possible adverse effects of red propolis

Application



IPRG

International Propolis
Research Group



5th International Propolis Conference

PROPOLIS: DEFENDER OF THE CITY

Rediscovering Natural Medicine

28th 29th & 30th May 2027

The Guildhall

York, England

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PROPOLIS: DEFENDER OF THE CITY

Rediscovering Natural Medicine

International Propolis Research Group





7TH INTERNATIONAL SYMPOSIUM ON BEE PRODUCTS & ANNUAL MEETING OF INTERNATIONAL HONEY COMMISSION

26-29 March 2026 // Poreč, Croatia





Thank you!

@apisflora



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Pharmaceutical-Biochemist, graduated from the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (FCFRP/USP), where she also obtained her Master's, PhD, and Postdoctoral degree in formulation technology with propolis.

R&D Leader at Apis Flora Indl. Coml. Ltda, where she has been working for 25 years. **Convenor elected by ISO member countries** to lead the creation of the international standard for propolis and propolis extract. **Board Member** of the International Propolis Research Group (IPRG). **Chair** of the Propolis Group of the International Honey Commission (IHC). **President of ABEMEL** for two terms, currently serving as **Vice-President**.