Sustainable extraction of bioactive compounds from *Eucalyptus globulus* leaves

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Introduction

Eucalyptus, a highly exploited tree from the Myrtaceae family, is the most represented tree in Portugal, accounting for 25.7% of the forest area, becoming crucial for the Portuguese pulp industry. There is a growing interest in extracting high-value bioactive compounds, such as phenolic compounds, from eucalyptus leaves due to their antimicrobial, antifungal, and antioxidant properties (Tiwari, 2015). These compounds have applications in food flavoring, sanitary products, and cosmetics. The exploration of eucalyptus for bioactive compounds aligns with the biorefinery concept, aiming for eco-friendly and zero-waste industrial processes, highlighting its significance in health, technology, and sustainability.

Therefore, this study aims to value eucalyptus (Eucalyptus globulus) leaves to obtain an extract rich in bioactive properties, using ultrasound-assisted extraction with a green solvent (ethanol:water) optimized through Response Surface Methodology combined with Genetic Algorithm.

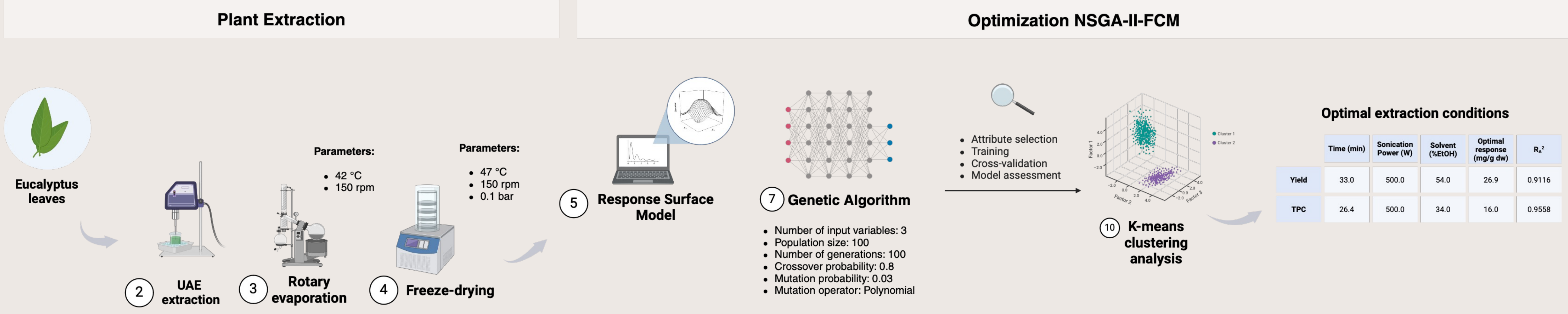
Methodology

Optimization method:

Response surface method created a multivariate model to approximate the objective function, and the genetic algorithm (GA) was then employed to navigate this approximated surface to find the optimal solution to maximize the yield (Y) and total phenolic content (TPC). GA mimics the process of natural selection by generating a population of solutions, evaluating their fitness, and using crossover and mutation operators to evolve better solutions over generations (Jha & Sit, 2021).

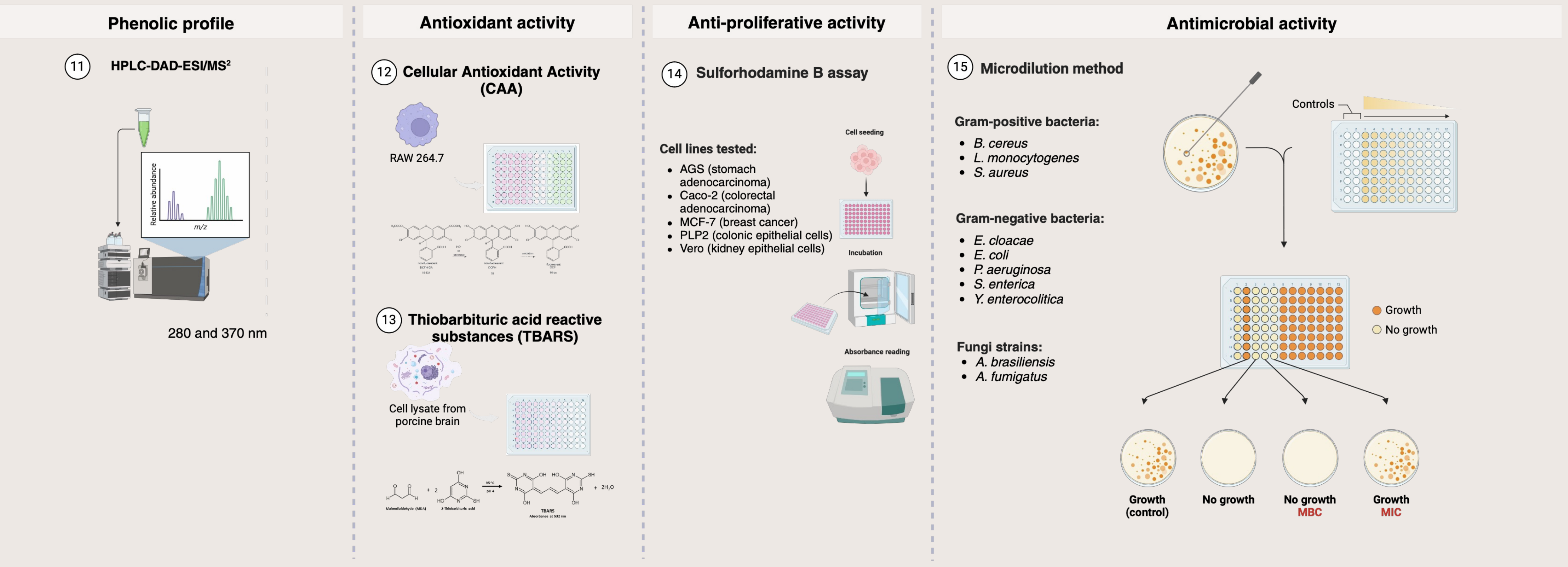
Bioactivity assays:

The antioxidant capacity was analyzed through two assays: Thiobarbituric Acid Reactive Substances Assay (TBARS) using porcine brain (Sus scrofa) tissue to mimic high-fat cells (Corrêa *et al.*, 2015), and Cellular Antioxidant Activity (CAA) using murine macrophage cells (De La Fuente *et al.*, 2022).. The antibacterial capacity was evaluated through the microdilution method in terms of minimum inhibitory concentration (MIC). The extracts were tested against food contaminants using a colorimetric assay previously described in De La Fuente *et al.* (2022).The anti-proliferative activity of the extracts was evaluated using the Sulforhodamine B (SRB) assay as described in De La Fuente *et al.* (2022).



Optimal extraction conditions

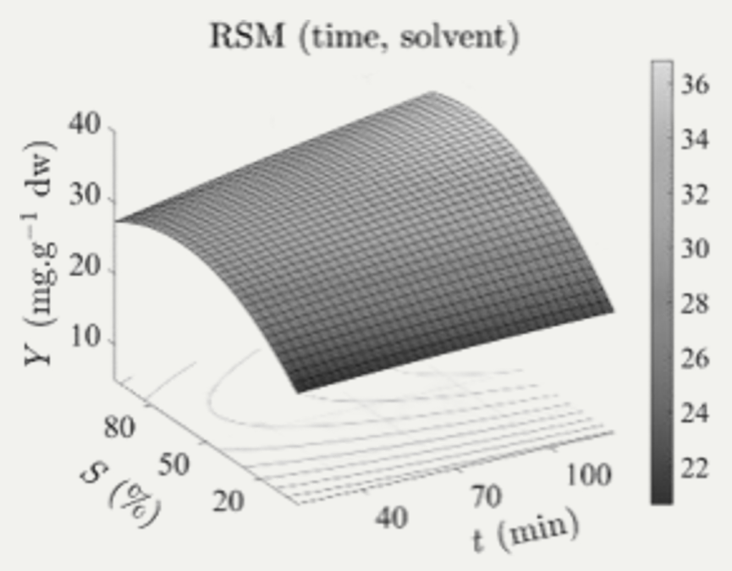
	Time (min)	Sonication Power (W)	Solvent (%EtOH)	Optimal response (mg/g dw)	R_A^2
Yield	33.0	500.0	54.0	26.9	0.9116
TPC	26.4	500.0	34.0	16.0	0.9558



Results

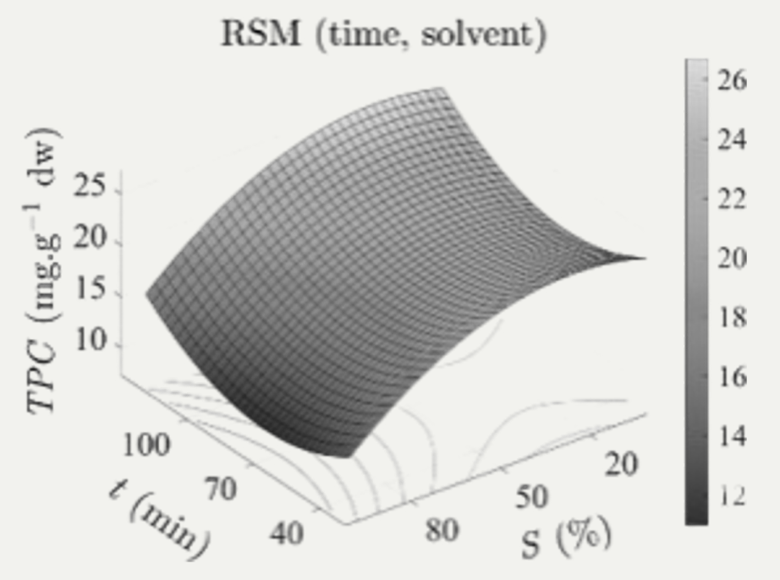
Optimization by RSM-GA

Extraction of bioactive compounds were most effective at high temperatures, long contact times, and higher ethanol concentrations, enhancing solubility, solvent diffusivity, and tissue permeability. UAE required less time and ethanol, being more cost-effective and sustainable, due to ultrasound-induced cavitation. However, prolonged cavitation can degrade phenolic compounds reducing extraction performance.



$$Y = 23.34 + 1.23t + 2.58P + 2.57\,S$$

$$TPC = 13.80 + 0.86P - 2.42\,S - 1.35\,S^2$$



Bioactivity of the extracts

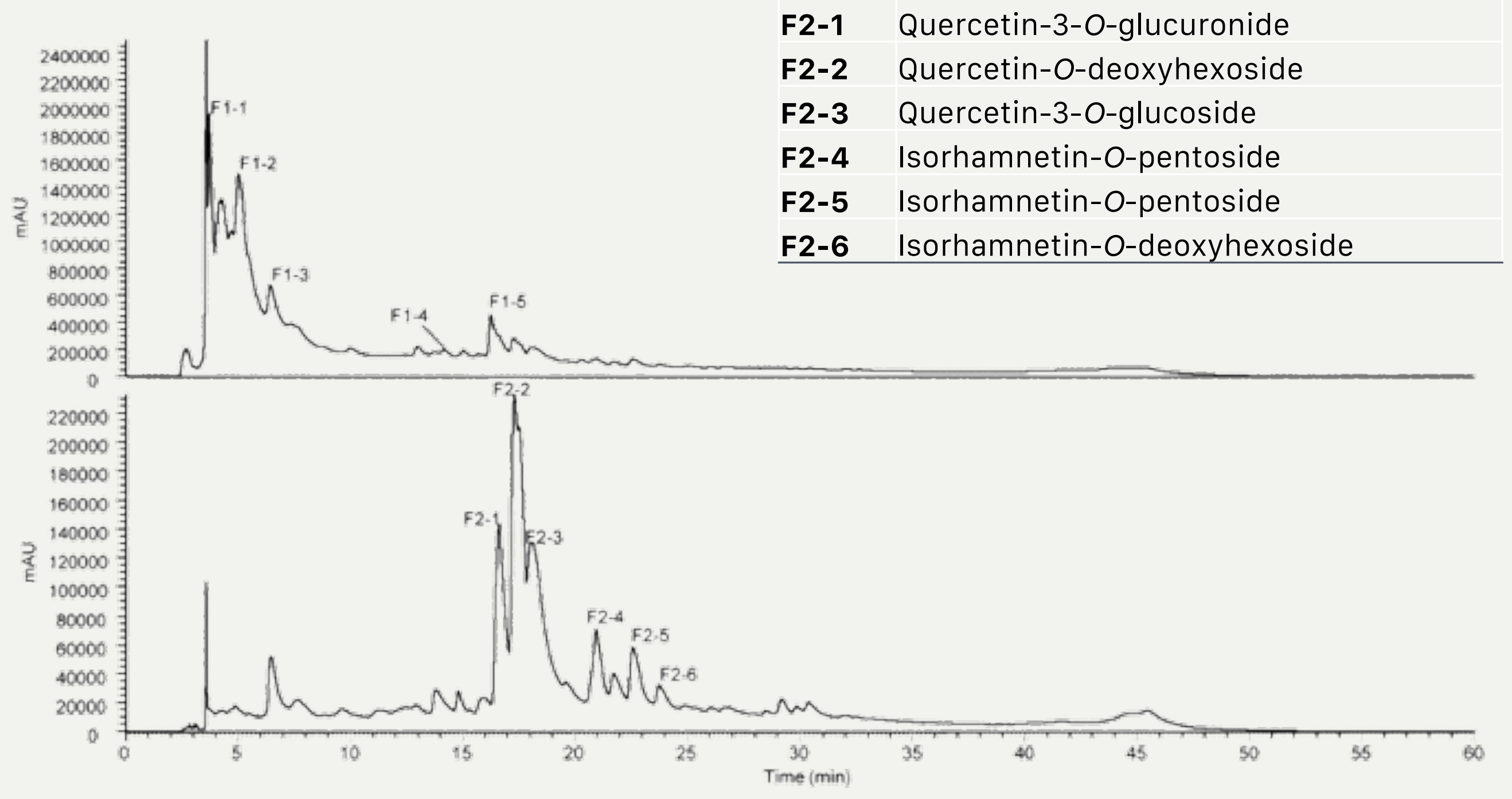
Table 3: EC_{50} values of the antioxidant and anti-inflammatory activities and GI_{50} for cytotoxicity.

	Extract		Positive control
	Y	TPC	(µg/mL)
Antioxidant activity			
TBARS (EC_{50}, mg/mL)	7.35 ± 0.55	4.87 ± 0.32	5.8 ± 0.6
CAA (% inhibition)	-	-	95 ± 5
Anti-proliferative activity			
AGS (GI_{50}, µg/mL)	58.6 ± 0.8	86.5 ± 6.2	1.2 ± 0.03
CaCo2 (GI_{50}, µg/mL)	65.0 ± 5.8	88.1 ± 7.3	1.2 ± 0.02
MCF-7 (GI_{50}, µg/mL)	48.1 ± 2.53	76.2 ± 5.14	1.0 ± 0.02
PLP2 (GI_{50}, µg/mL)	42.4 ± 3.94	64.9 ± 0.85	1.4 ± 0.10
Vero (GI_{50}, µg/mL)	54.4 ± 0.62	73.3 ± 1.80	1.4 ± 0.06

Controls: Quercetin (CAA), Ellipticine (anti-proliferative assay).

Phenolic profile and quantification by chromatographic analysis

Peak	Tentative Identification
F1-1	Digalloyl-glucose
F1-2	Digalloyl-glucose
F1-3	5-*O*-Caffeoylquinic acid
F1-4	Gallotannin
F1-5	Eucaglobulin/Globulusin B
F2-1	Quercetin-3-*O*-glucuronide
F2-2	Quercetin-*O*-deoxyhexoside
F2-3	Quercetin-3-*O*-glucoside
F2-4	Isorhamnetin-*O*-pentoside
F2-5	Isorhamnetin-*O*-pentoside
F2-6	Isorhamnetin-*O*-deoxyhexoside



Conclusions

- *E. globulus* has proven to be an important source of phenolic compounds, rich in phenolic acids and flavonoids, with antibacterial, antifungal, anti-inflammatory, and antioxidant activities, presenting low cell toxicity.
- The results showed that the extracts with maximum total phenolic content presented the highest bioactivity. The study highlights the importance of using multiple assays to analyze the antioxidant potential of plant extracts and suggests that different classes of compounds may work synergistically for this mechanism.
- Extracts of *E. globulus* proved to be a potential natural additive with many applications in the food industry, mainly as a natural preservative and flavoring agent. Its antimicrobial and antioxidant properties, as well as its distinctive taste and potential health benefits.

The exploration of the potential of eucalyptus leaf extract as a bioactive agent proposed in this work is directly linked to the SDGs "3: Health and Well-being" and "12: Responsible Consumption and Production", since the use of these agro-industrial residues, the The use of low toxicity solvents and low environmental impact extraction methods promote the sustainable production of natural additives that can improve public health and reduce the environmental impact of the food industry.

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