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Research Article

Unlocking the Full Potential of Green Propolis: A Novel Extraction Approach Using Eutectic Solvents for Improved Phenolic Compound Recovery

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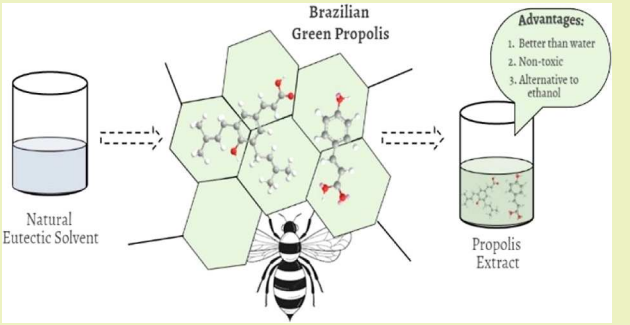
ABSTRACT: Propolis is highly diverse and has various health benefits for humans. However, standardizing propolis-based products for use in the pharmaceutical and food industries is challenging. Efficient extraction processes are essential to maximize their biological potential. Ethanol is commonly used as a solvent but has several drawbacks, such as residual flavor and limitations for some individuals. Water is an alternative, but it is not as effective as ethanol. In this context, new strategies are being explored to find an alternative to ethanol and improve water's performance as a solvent. To this end, a new extraction platform of phenolic compounds from Brazilian green propolis using eutectic solvents was developed. Betaine: citric acid (1:2) with 50 wt % of water was found to be the most efficient solvent, with a yield of extraction around 100 $mg_{polyphenol}$ $g_{propolis}^{-1}$ under optimum conditions with an ultrasound-assisted extraction approach (UAE). In vitro biological assays were performed to evaluate the potential of this new extract, mainly regarding antioxidant, antihypertensive, lipase inhibition, and antimicrobial activity. Our results indicate that the eutectic solvent selected in the extraction process enhances the biological potential of the green propolis extract, opening the doors for the development of semi-finished products active against inflammation biological pathways.



KEYWORDS: *Brazilian propolis, natural formulation, polyphenols, alternative solvents, biological assays*

INTRODUCTION

Propolis ("bee glue") is a natural resinous mixture produced by different bee species, including honeybees and stingless bees.[1] Propolis consists of plant resin and balsam (50%), waxes (30%), essential and aromatic oils (10%), pollen (5%), and other organic compounds (5%). More than 850 constituents were already identified in the propolis composition, depending on the propolis origin.[1−3] It is also classified considering its physical−chemical characteristics, vegetal source, bee species, geographic origin, and chemical composition. The color of propolis varies from brown to yellow, red, and green.[1,2]

Propolis has many therapeutic benefits for humans, such as antibacterial, antifungal, antitumor, antioxidant, and anti-inflammatory.[4−6] As a result, there is a growing interest in developing and optimizing new formulations containing propolis that can preserve or even improve its properties and biological potential. But, considering its biodiversity, the standardization of propolis, a specialty chemical, is an obstacle to its extensive use.[7]

The European Food Safety Authority (EFSA) stated that establishing a relationship between propolis consumption and health effects is likely.[8] However, selecting the right propolis and extraction process is necessary due to variations in phenolic compounds present.[9,10] Thus, to develop an efficient extraction process while preserving their biological properties, it is crucial to use an appropriate solvent to solubilize the biomass and recover the desired compounds in a high-performance and sustainable operational mode.[11]

Ethanol is the primary solvent used to produce propolis extracts on a large scale.[2,7,12] Unfortunately, ethanol has some drawbacks, such as residual flavor and inadequate application in some medical fields, including ophthalmology and pediatrics.[13,14] Additionally, it is not recommended for specific populations, such as Muslims, pregnant women, children, and people with alcohol intolerance.[15,16] Water is often used as an alternative solvent to ethanol, but it does not have an equivalent extraction performance.[2,7,12] Therefore, new strat-

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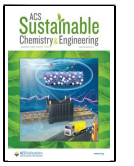

egies must be explored, considering the search for new solvents.

Eutectic solvents,[17] also known as deep eutectic solvents (DESs), are a promising and sustainable medium for extracting natural products, such as propolis compounds.[18−22] DES are versatile solvents with numerous possibilities,[23] prepared by mixing a hydrogen bond acceptor with at least one hydrogen bond donor in a specific stoichiometric ratio.[17] Although DESs have gained interest as an alternative solvent for propolis extraction, few studies have evaluated their efficiency and safety compared to other solvents.[18−22] Additionally, researchers suggest that DES components may enhance the biological activity of extracted compounds.[20,24] Therefore, this article sought to innovate by choosing eutectic solvents that can function not only as solvents but also as active ingredients, thereby increasing the functionality of the final extract. Along with this, the method created here focused on minimizing the environmental impact associated with the use of volatile organic solvents. It is important to note that most studies investigating the use of eutectic solvents to extract bioactive compounds from propolis generally neglect environmental analysis, which is crucial to assess the overall sustainability of the developed process compared to existing methods.[11,25]

This study aims to investigate the DES potential as a solvent and an efficient biological additive in the extract. Several biological assays are included to evaluate the biological potential of the DES-based extracts compared to those obtained using ethanol or water as solvents. The biological assays included are critical in preventing diseases related to inflammation pathways, such as cancer, obesity, and COVID-19. The study highlights the importance of developing innovative methods for producing high-quality natural products while meeting the current market's demands and the Sustainable Development Goals.[26]

EXPERIMENTAL SECTION

Samples and Materials. Mn Propolis (Mogi das Cruzes-SP, Brazil) kindly donated the green raw propolis. The frozen samples were briefly blended in a domestic blender (Model OSTER, 450 W 220 V, São Paulo, Brazil). The resulting milled propolis samples were sifted through a steel sieve (Model Bestifer, Limeira, Brazil) to standardize the particle size between 0.5 and 1 mm. The samples were then stored in a dark container at −20 °C until use. All the compounds required to prepare the eutectic solvents were purchased from Dinâmica (Campinas, Brazil). These included cholinium chloride (ClCh; >96%), betaine (Bet; >98%), proline (Pro; 100%), ammonium acetate (AmmAc; >95%), 1,4-butanediol (But; >98%), 1,2-ethylene glycol (Ety; >98%), citric acid (CA; >99%), malic acid (MA; 100%), acid acetic (AA; >99%), lactic acid (LA; 85%), and sorbitol (Sorb; >99%).

Ethanol absolute (EtOH) was purchased from Synth (Diadema, Brazil). Ultrapure water was obtained from a Purelab Flex 3 purifying system (ElgaVeolia, High Wycombe, United Kingdom). Acetonitrile purchased from J.T. Baker was used for the chromatographic analysis. The chromatographic standards, *p*-coumaric acid (*trans*-4-hydroxycinnamic acid, ≥98.0%) and artepillin C (2*E*)-3-[4-hydroxy-3,5-bis(3-methyl-2-butene-1-yl) phenyl]-2-propenoic acid, ≥90.0%, were purchased from Sigma-Aldrich Ltda (São Paulo, Brazil). A fused-core type column (KinetexC-18, 50 × 72.1 mm, particle size 1.3 μm; Phenomenex, Torrance, CA, USA) was used for UPLC-PDA analysis.

COSMO-RS Model. This study used the COSMO-RS, a quantum chemistry-based thermodynamic model, to determine the most effective DES for extracting phenolic compounds from green propolis. The Turbomole software (TmoleX19 Version 4.5) was used to optimize the geometry and charge density of the molecules (COSMO-BP-TZVP) for the COSMO-RS calculations. The COSMO-RS calculations were carried out using the COSMOtherm package (Version 21.0) with the BP_TZVP_21 parameterization.[27] A total of 10,585 samples were designed by combining 73 hydrogen bond acceptors (HBAs) and 145 hydrogen bond donors (HBDs) to screen DESs. The most promising combinations of HBA and HBD for extracting phenolic compounds were selected based on the activity coefficient values of the phenolic model compounds in different DES at infinite dilution, represented by the model molecules artepillin C and *p*-coumaric acid. Tables S1 and S2 in the Supporting Information contain the list of HBAs and HBDs used in the study.

Preparation of Eutectic Solvents. A method adapted from Abbott and co-workers[17] was used to prepare the eutectic solvents, resulting in the experimental preparation of 32 eutectic mixtures and 11 isolated components (500 mM), each containing 20% (w/w) of water based on the starting material's initial water content. The initial molar ratio used was 1:2 (following the method described by ref 28) of HBA and HBD components that were weighed and added to a closed glass flask, stirred at 60 ± 2 °C and 500 rpm in an oil bath until a homogeneous and transparent liquid was obtained, and then stored for 24 h before use.

Solid−Liquid Extraction. The capacity of various eutectic solvents to extract polyphenol compounds from crude green propolis samples was evaluated by determining the yield of total polyphenols ($mg_{polyphenol}\ g_{propolis}^{-1}$) using ultra-performance liquid chromatography coupled with a photodiode array detector (UPLC-PDA, Waters Corp, Acquity H-Class, Milford, Massachusetts, USA) coupled with mass spectrometry (Thermo Fisher Scientific LTQ XL linear ion trap mass spectrometer, San Jose, California, USA), following the literature.[29] The extractions were performed in triplicate, and the mean ± standard deviation data was calculated.

Initial Screening. The initial screening involved ultrasonic-assisted extraction using an ultrasonic bath (P60H, Elmasonic, Singen, Germany, 2.75 L, 37 kHz, 135 W) under fixed operational conditions (namely, the solid−liquid ratio [$R_{(S/L)}$] of 0.015, meaning 0.015 g of propolis per mL of solvents for 120 min (min) at 55 °C), according to the method described by (Contieri et al., 2022).[29] After extraction, a 5 mL aliquot was centrifuged at 14,000 rpm and 20 °C for 15 min. The supernatant was then collected and diluted using a dilution factor of 4× in water and filtered through nylon filter membranes with a pore size of 0.22 μm (Nova Analitica Imp. Exp. Ltda, São Paulo, Brazil) before UHPLC-PDA analysis. The yields were compared with those obtained using pure ethanol (EtOH 100% v/v) and water (100% v/v) as controls.

Step-by-Step Optimization of Operational Parameters. After selecting the most promising eutectic solvent, the operation parameters were optimized using a step-by-step strategy in an ultrasonic bath (37 kHz, 135 W) for 120 min, with an $R_{(S/L)}$ of 0.015 and a fixed temperature at 55 °C. The optimized conditions included HBA/HBD molar ratios of 3:1, 2:1, 1:1, 1:2, and 1:3 and eutectic solvent water percentages of 20, 25, 30, 35, 40, 50, and 60% (w/w).

Optimization Using Ultrasonic Probes. The ultrasonic bath was replaced by an ultrasonic probe (Ultronique, 800 W, 20 kHz), starting with 3 min of extraction time to maximize the yield and reduce the screening test time (120 min).[30] A central composite rotatable design (CCRD 2^3) was then performed, with 3 central points as replicates, totaling 11 trials. This design was based on 3 independent operational variables: $R_{(S/L)}$—0.02, 0.03, and 0.04; cavitation power (W)—240, 320, and 400; and static time (t_{sta})—5, 10, and 15 min (contact time between sample/solvent before extraction without cavitation). The responsive variable was the yield of polyphenols recovered from green propolis, expressed as $mg_{polyphenols}\ g_{propolis}^{-1}$, and the optimum conditions were validated by performing three extractions under the selected conditions.

The observed results were compared with those predicted by the model. The respective variation coefficient was expressed as a percentage of the expected versus observed values. The extraction time was further optimized under the selected operational conditions by testing six different times (1, 3, 6, 9, 12, and 15 min), and the extraction yield of polyphenol compounds ($mg_{polyphenols}\ g_{propolis}^{-1}$) from propolis was determined. After optimization using the ultrasonic

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probe, conventional solvents, such as ethanol (100% v/v) and water (100% v/v), were tested and compared with the eutectic solvent.

Comparison among Extraction Techniques. The performance of the ultrasonic probe extraction optimized method was also compared with other extraction techniques tested in this work, including ultrasonic bath ($R_{(S/L)}$ of 0.02, 150 W, 6 min), magnetic stirring ($R_{(S/L)}$ of 0.02, 185 W, 6 min, 500 rpm), and shaker ($R_{(S/L)}$ of 0.02, 6 min, 80 rpm). All extractions were performed in triplicate, and the mean ± standard deviation ($mg_{polyphenols}\ g_{propolis}^{-1}$) was calculated.

Quantification of Polyphenols. To quantify the propolis polyphenols in the extracts, we used the method proposed by ref 29. The extracts were analyzed using ultra-high performance liquid chromatography coupled with a photodiode array detector (UPLC-PDA, Waters Corp, Acquity H-Class, Milford, MA, USA) coupled with mass spectrometry (Thermo Fisher Scientific LTQ XL linear ion trap mass spectrometer—San Jose, CA, USA). The separation was achieved with a fused-core column (Kinetex C-18, 50 × 2.1 mm, 1.3 μm: Phenomenex, Torrance, CA, USA), with the temperature fixed at 55 °C, and a mobile phase flow of 0.5 $mL_{solvent}\ min^{-1}$.

Extract Characterization. *ABTS Assay.* The ABTS scavenging assay was performed using a 96-well microplate with the modifications introduced by.[31] The concentration of ABTS radical was adjusted with water to an initial absorbance of 0.700 (±0.020) at 734 nm. In each microplate well, 280 μL of the ABTS solution was mixed with 20 μL of either the sample, Trolox, or solvent. The mixture was then incubated at 30 °C for 5 min, and the absorbance was measured at 734 nm using a multidetection plate reader (Synergy H1, VT, USA). The analyses were performed in triplicate, expressing the results as Trolox equivalents. A solid−liquid ratio of 0.02 was used, and the scavenging activity was presented as the percentage reduction in absorbance with respect to the control. Regression equations were determined between net ABTS scavenging and Trolox concentration, and the values were expressed as Trolox equivalents using the standard curve calculated for each assay. The results are reported in $\mu mol_{TroloxEquivalent}\ mL_{propolisextract}^{-1}$.

Oxygen-Radical Absorbance-Capacity Assay. The oxygen-radical absorbance-capacity (ORAC)-fluorescein assay was performed in a black 96-well microplate (Nunc, Denmark), using a method described by ref 32 with some modifications. The reaction was carried out in a 75 mM phosphate buffer (pH 7.4), and the final reaction mixture volume was 200 μL. Antioxidant (20 μL) and fluorescein (FLC) (120 μL; final concentration 70 mM) solutions were added to the microplate wells. Each assay included a blank (FL + AAPH—(2,2-azobis(2-amidinopropane) dihydrochloride)) using phosphate buffer instead of the antioxidant solution, and eight calibration solutions using Trolox (final concentration of 1−8 μM) as an antioxidant were prepared. After preincubation for 10 min at 37 °C, the AAPH solution (60 μL; final concentration 12 mM) was added using a multichannel pipette. The microplate was immediately placed in the reader, and the fluorescence was recorded at intervals of 1 min over 140 min. The ORAC-fluorescein assay was performed using a multidetection plate reader (Synergy H1, VT, USA) with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. A solid−liquid ratio of 0.02 g mL^{-1} was used for this analysis. The reaction was carried out in triplicate, and the results were expressed in $\mu mol_{TroloxEquivalent}\ mL_{propolisextract}^{-1}$.

ACE-Inhibitory Activity Assay. The ACE inhibitory activity was measured using fluorescence with modifications to the method described by.[33] Ultrapure water or ACE working solution (40 μL) was added to each well of a microtiter plate, then adjusted to 80 μL by adding ultrapure water to a blank (BLK), control (CTL), or sample (SPL). For direct or 1/2 diluted samples, a sample blank (SPLB) was also prepared. The enzyme reaction was initiated by adding 160 μL of the substrate solution, and the mixture was incubated at 37 °C. Serial dilutions of each sample were made from 1/1 to 1/32. After 30 min, the fluorescence generated was measured using a multidetection plate reader (Synergy H1, VT, USA). The assay was performed in a black 96-well microplate (Nunc, Denmark), with excitation and emission wavelengths of 350 and 420 nm, respectively. The solid−liquid ratio for this analysis was 0.02. The inhibitory activity was expressed as the peptide concentration required to inhibit the original ACE activity by 50% (IC 50).

Pancreatic Lipase Inhibition Assay. The pancreatic lipase inhibition assay was performed in a previous study[34] with some modifications. The porcine pancreatic lipase enzyme [type II, Sigma-Aldrich Ltda (São Paulo, Brazil)], at a concentration of 10 g L^{-1}, was prepared in Tris−HCl 0.05 mol L^{-1}, pH 8.0 (containing $CaCl_2$ 0.010 mol L^{-1} and NaCl 0.025 mol L^{-1}). *p*-Nitrophenylpalmitate (lipase substrate) 0.008 mol L^{-1} was dissolved in Triton-X 100, 0.5% (w/v), at 37 °C. All analyses were performed in triplicate. In each analysis, the reaction mixture (100 μL of the enzyme, 50 μL of water, and 50 μL of the substrate) was incubated for at least four different periods. Controls without enzyme (substrate blank) and without substrate (enzyme blank) were incubated in the same way as the experimental tubes. *p*-Nitrophenol (product of the action of the lipase on the *p*-nitrophenol palmitate) of yellow colors in the assay pH was read at 410 nm. The slope of the graph line, absorbance vs time, was followed and used as a reference for calculating the % passage and the inhibited lipase activity. Inhibition was obtained from the slope of the line graph absorbance vs time of the tests in the absence of the extract (A), in the absence of the extract and enzyme (substrate blank − a), in the presence of the extract, enzyme, and substrate (B), and in the absence of the enzyme (blank extract + substrate − b) in the same way as the lipase activity assay (100 μL of enzyme, 50 μL of extract, and 50 μL of substrate). In each analysis, the extracts were pre-incubated with the enzyme for 10 min before adding the substrate and starting the time count. The percentage of inhibition (I) was calculated by eq 1.

$$I\ (\%) = (A - a) - (B - b)/(A - a) \times 100 \quad (1)$$

The results were presented as % inhibition of lipase activity per mL of propolis extract (% ALI/mL of propolis extract), indicating the amount of 1 μmol *p*-nitrophenol that remained unproduced per minute due to the presence of the during the test.

Microorganisms and Culture Conditions. Different strains and incubation conditions were employed to evaluate the antibacterial activity of propolis extracts. Six microorganisms were evaluated, including *Staphylococcus aureus*; *Escherichia coli*; methicillin-resistant *S. aureus* (MRSA); methicillin-sensitive *S. aureus* (MSSA); *Staphylococcus epidermidis,* and *Candida albicans*. These strains were cultured overnight at 37 °C in a culture medium using specific incubation conditions. The cultures were diluted with a culture medium to achieve a final absorbance (OD_{630}) of 0.1, corresponding to approximately 10^8 cfu mL^{-1}, which was used for the antibacterial activity experiments. In the ethanolic extracts, the ethanol was completely evaporated and then resuspended in DMSO.

Bacterial Growth Inhibition. To assess bacterial growth inhibition, a flat-bottomed 96-well microplate was used. Each well, except for the first row, which served as a blank, was loaded with 10 μL of bacterial culture. Subsequently, 10 μL of each extract concentration was added to all wells to obtain a final concentration of 0.002 g mL^{-1}. Two controls were used in two columns of the microplate: one containing 10 μL of diluted bacterial strains and sterile distilled water as a positive control, and the other containing 10 μL of the culture medium without bacterial culture as a negative control. The bacterial growth inhibition was determined by measuring the optical density at 650 nm using a multidetection plate reader (Synergy H1, Vermont, USA). The percentage inhibition was calculated using eq 2

$$\text{inhibition}\ (\%) = (OD_{\text{control bacteria}} - OD_{\text{bacteria/control bacteria}}) \times 100 \quad (2)$$

$OD_{controlbacteria}$ and $OD_{bacteria}$ represent the optical density at 650 nm after 24 h of incubation of the bacteria without and with the propolis extract powder, respectively. The tests were performed in duplicate. The bacterial growth inhibition was expressed as IC_{50}, defined as the concentration ($g_{propolis}\ mL^{-1}$) of the antibacterial compound required to achieve a 50% inhibition of bacterial growth after 24 h.

Environmental Analysis—EcoScale. The environmental analysis was conducted using the EcoScale database,[35] which assigned

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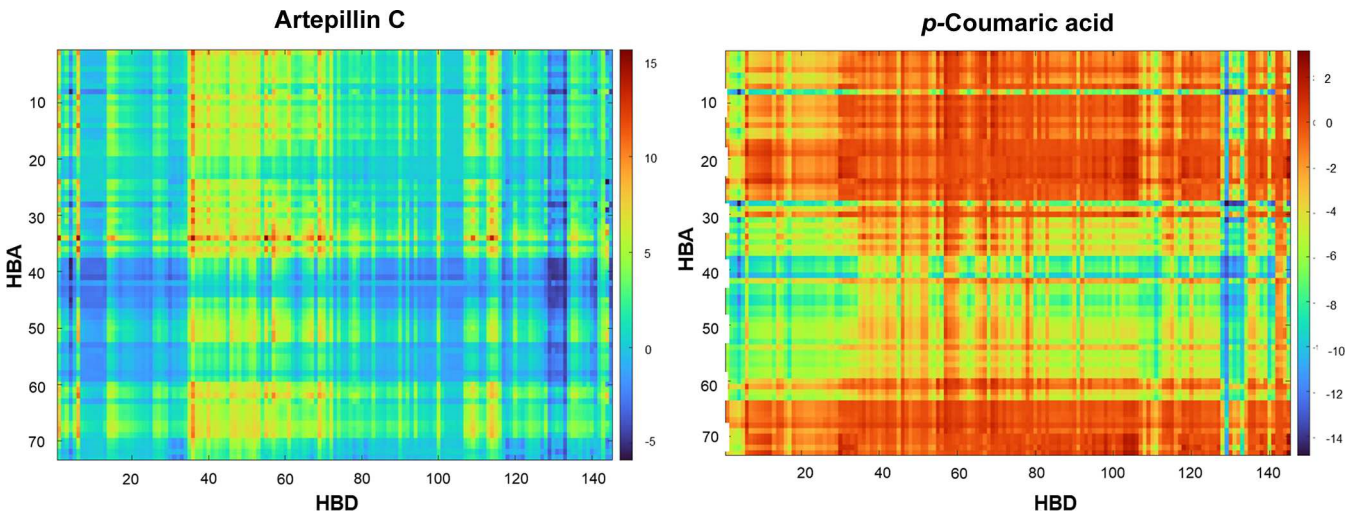

Figure 1. Activity coefficients at infinite dilution (ln γ^{∞}) of artepillin C (left) and *p*-coumaric acid (right) using different eutectic solvents (1:2) at 308.15 K.

Table 1. Chemical Name, Abbreviation, Molar Mass, and Purity of the Eutectic Solvents Used in the Initial Screening

DES formulation (1_{HBA}:2_{HBD})	abbreviation	MM (HBA)[a]	MM (HBD)[a]	purity HBA (%)	purity HBA (%)
cholinium chloride/1,4-butanediol	ChCl/But	139.62	90.12	96	98
cholinium chloride/1,2-ethylene glycol	ChCl/Ety		60.07		99
cholinium chloride/citric acid	ChCl/CA		192.12		≥99
cholinium chloride/sorbitol	ChCl/Sorb		182.17		99
cholinium chloride/lactic acid	ChCl/LA		90.08		85
cholinium chloride/malic acid	ChCl/MA		134.08		98
betaine/1,4-butanediol	Bet/But	117.15	90.12	98	98
betaine/1,2-ethylene glycol	Bet/Ety		60.07		99
betaine/citric acid	Bet/CA		192.12		≥99
betaine/sorbitol	Bet/Sorb		182.17		99
betaine/lactic acid	Bet/LA		90.08		85
betaine/malic acid	Bet/MA		134.08		98
betaine/acetic acid	Bet/AA		60.05		≥99
proline/1,4-butanediol	Pro/But	115.13	90.12	100	98
proline/1,2-ethylene glycol	Pro/Ety		60.07		99
proline/citric acid	Pro/CA		192.12		≥99
proline/lactic acid	Pro/LA		90.08		85
proline/acetic acid	Pro/AA		60.05		≥99
proline/sorbitol	Pro/Sorb		182.17		99
ammonium acetate/1,4-butanediol	AmmAc/But	77.08	90.12	95	98
ammonium acetate/1,2-ethylene glycol	AmmAc/Ety		60.07		99
ammonium acetate/acetic acid	AmmAc/AA		60.05		≥99
ammonium acetate/lactic acid	AmmAc/LA		90.08		85

[a]MM: molar mass (g mol^{-1}).

penalty points based on (i) safety, (ii) solvent, (iii) price/availability for industrial applications, (iv) technical setup, (v) temperature applied on the extraction process, and (vi) workup and purification steps. All these factors were normalized by the yield of extraction (%) of polyphenols from green propolis. The results were presented as an EcoScale score, ranging from 1 to 100, where 1 represents the least, and 100 represents the most environmentally friendly process. A comparison was made between the unit operations of maceration, magnetic stirring, shaker, ultrasonic bath, and ultrasonic probe methods.

Statistical Analysis. Analysis of variance (ANOVA) followed by the Bonferroni posthoc test was used to compare the extraction yield of different extraction unit operations and determine the optimal extraction time. The extraction yield of different extraction solvents was also compared. The significance level was 95% ($p < 0.05$, $n = 3$). All analyses were conducted using JAMOVI (Version 2.3) Computer Software.

RESULTS AND DISCUSSION

Initial Screening of DESs. Propolis samples owe their health benefits to the polyphenol compounds they contain,[7] with flavonoids, lignans, stilbenes, phenylpropanoids, caffeoylquinic acid derivatives, and hydroxycinnamic acid derivatives being the most abundant.[2,7,36] Among these compounds, *p*-coumaric acid and artepillin C are the chemical markers of green propolis polyphenols, making them suitable models for in-silico screening.

The screening was started by calculating the activity coefficient at infinite dilution (ln γ^{∞}) of artepillin C and *p*-

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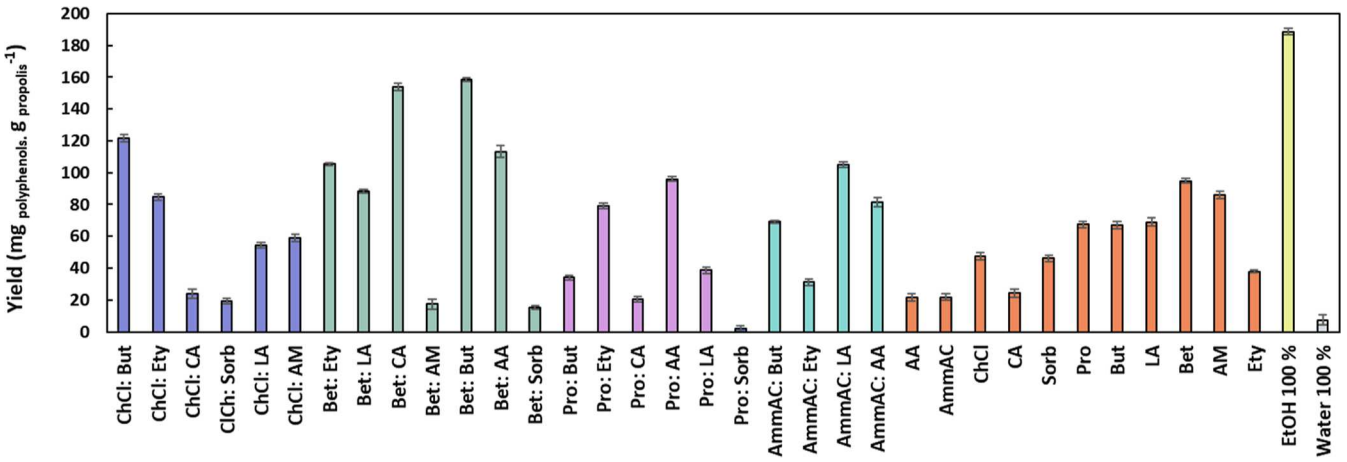

Figure 2. Results of the yield of extraction of polyphenols from green propolis using 25 eutectic mixtures (20 wt % and molar ratio 1:2), the 11 starting materials (500 mm) by UAE (135 W, $R_{(S/L)}$ 0.015 for 120 min), and pure water and ethanol (EtOH), used as controls. Abbreviated names: cholinium chloride (ChCl), betaine (Bet), proline (Pro), ammonium acetate (AmmAc), 1,4-butanediol (But), 1,2-ethylene glycol (Ety), citric acid (CA; >99%), malic acid (MA; 100%), acetic acid (AA; >99%), lactic acid (85%), and sorbitol (Sorb; >99%). The bars show the extraction yield in $mg_{polyphenol}\ g_{propolis}^{-1}$ with the standard deviation of the extraction made in triplicate.

coumaric acid in 10,585 combinations of HBAs and hydrogen bond donors (HBDs). As the COSMO-RS was used as an initial screening tool, it was decided to use a molar ratio close to 1:1 (1:2). As can be seen in Figure S1 (Supporting Information), the trends obtained for the different molar ratios (1:1 and 1:2) were similar. Figure 1 shows the results, with the vertical axis representing the 73 HBAs tested and the horizontal axis representing the 145 HBDs. The ln γ^{∞} value is represented by the color of each point in the graph, with a smaller (more negative) value indicating greater solvent solvation capacity and higher values representing lower solvent dissolution capacity.[37] Eutectic solvents with lower ln $\gamma\infty$ values and more significant dissolution potential appear in the dark blue areas, while solvents with lower capacities appear in the red/yellow areas. Although artepillin C and *p*-coumaric acid exhibited similar trends, *p*-coumaric acid had smaller ln $\gamma\infty$ values than artepillin C, suggesting that eutectic solvents have a greater capacity to dissolve *p*-coumaric acid.

Among the HBAs tested in combination with different HBDs such as carboxylic acids [acetic acid (**56**) lactic acid (**62**), formic acid (**55**)] or alcohols [ethylene glycol (**15**), glycerol (**14**), 1,4-butanediol (**26**), and 1,6-hexanediol (**20**)] as HBDs, betaine (**8**), cholinium acetate (**28**), ammonium acetate (**30**), and cholinium chloride (**32**) exhibited the highest dissolution potential (Tables S1 and S2 in the Supporting Information). In contrast, eutectic mixtures containing sugars such as xylitol, sorbitol, sucrose, and HBD fatty acids like octanoic and nonanoic acids showed lower dissolution capacity for the model compounds. Based on the COSMO-RS predictions, we tentatively prepared 32 different aqueous solutions of eutectic solvents with 20% of water and an HBA/HBD molar ratio of 1:2. However, only 25 eutectic solvents were formed and used in extraction tests. A solid−liquid ratio $R_{(S/L)}$ of 0.015 g of propolis per mL of solvent was used to extract phenolic compounds from propolis.

Table 1 shows the list of DES components studied, and Figure 2 shows the extraction yields obtained from aqueous solutions of different eutectic solvents and their respective starting materials (HBAs and HBDs) on the extraction of polyphenols from propolis using UAE (135 W, $R_{(S/L)}$ = 0.015 for 120 min). The extraction yields from pure water and ethanol (EtOH), used as controls, are also presented. The results showed that while eutectic solvents improved the extraction yield obtained with pure water (with the lowest value, 7 ± 1 $mg_{polyphenol}\ g_{propolis}^{-1}$), none of the eutectic solvents could surpass the extraction performance of ethanol (189 ± 3 $mg_{polyphenol}\ g_{propolis}^{-1}$). Nonetheless, eutectic solvents composed of Bet/CA (betaine/citric acid; 1:2 and 20 wt % of water) and Bet/But (betaine/1,4-butanediol, 1:2 and 20 wt % of water) were the mixtures promoting the highest extraction yields (Bet/CA, 154 ± 3 $mg_{polyphenol}\ g_{propolis}^{-1}$; Bet/But, 158 ± 4 $mg_{polyphenol}\ g_{propolis}^{-1}$), indicating the synergistic effect of the compounds on the extraction efficiency.

Individually analyzed betaine 95 ± 2 $mg_{polyphenol}\ g_{propolis}^{-1}$, citric acid 24 ± 2 $mg_{polyphenol}\ g_{propolis}^{-1}$, and 1,4-butanediol 67 ± 3 $mg_{polyphenol}\ g_{propolis}^{-1}$ could not surpass the highest extraction yields obtained by their respective DES. The most promising eutectic solvents, Bet/AC and Bet/But, were analyzed, and in both cases, betaine was the best HBA, which is in accordance with COSMO-RS trends. Figure S2 (Supporting Information) compares the polyphenol extraction yields and ln γ^{∞} for the different eutectic mixtures. Eutectic solvents have been previously used for propolis phenolic extraction.[18−22]

Although using betaine-based solvents is uncommon, it is an excellent choice since it is approved for human consumption by the Food and Drug Administration (FDA) under section 505 of the Federal Food, Drug, and Cosmetic Act. The best combination with betaine, regarding HBDs, was found to be citric acid and 1,4-butanediol. Citric acid is a weak organic acid commonly found in citrus fruits and widely used in the food industry and others (INS 330 acidulant),[38] as a natural preservative, acidulant, flavor enhancer, buffering agent, and stabilizer.[39]

On the other hand, 1,4-butanediol is a non-natural alcohol used as a chemical reagent or intermediate in textiles, plastics, cosmetics, medicine, and chemistry.[40] While it is not considered dangerous, it does not offer the same health benefits as citric acid. The combination of betaine and citric acid is a powerful alternative to water and ethanol, providing health benefits for the obtained extract. As a result, Bet/CA (1:2 and 20 wt % of water) was chosen as the solvent to continue the study and optimize the operating conditions. This

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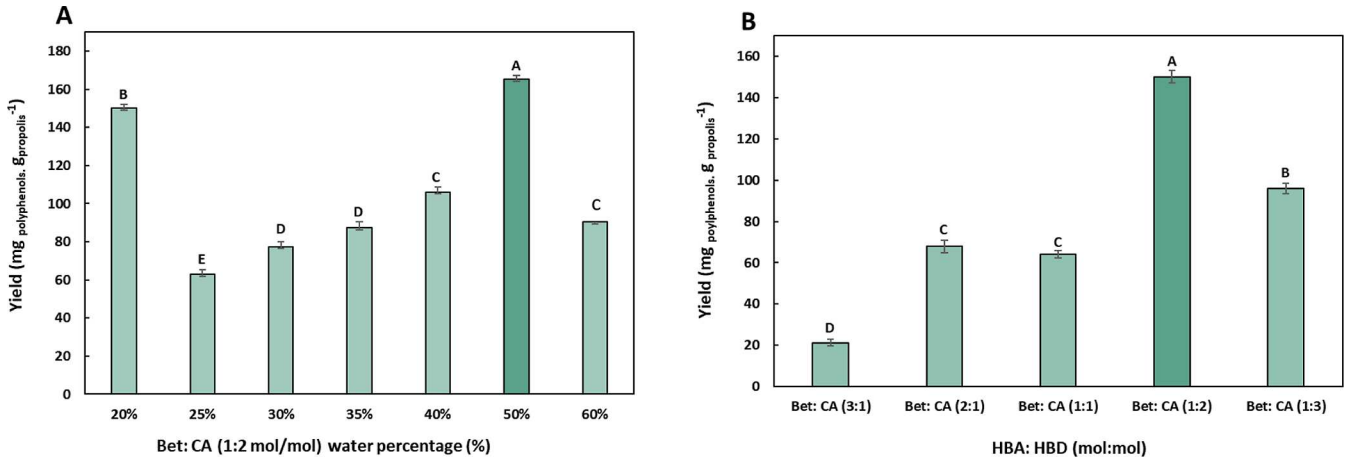

Figure 3. Effect of different parameters in extracting polyphenols from green propolis using an ultrasonic bath (37 kHz, 135 W, 45 °C for 2 h) and an $R_{(S/L)}$ of 0.015. (A) Eutectic solvents (1:2) prepared with different water percentages (20, 25, 30, 35, 40, 50, and 60 wt %). (B) Bet/CA at different molar ratios of 3:1, 2:1, 1:1, 1:2, and 1:3 + 20 wt % of water. Different letters show significantly different statistical results (ANOVA, p-value < 0.05).

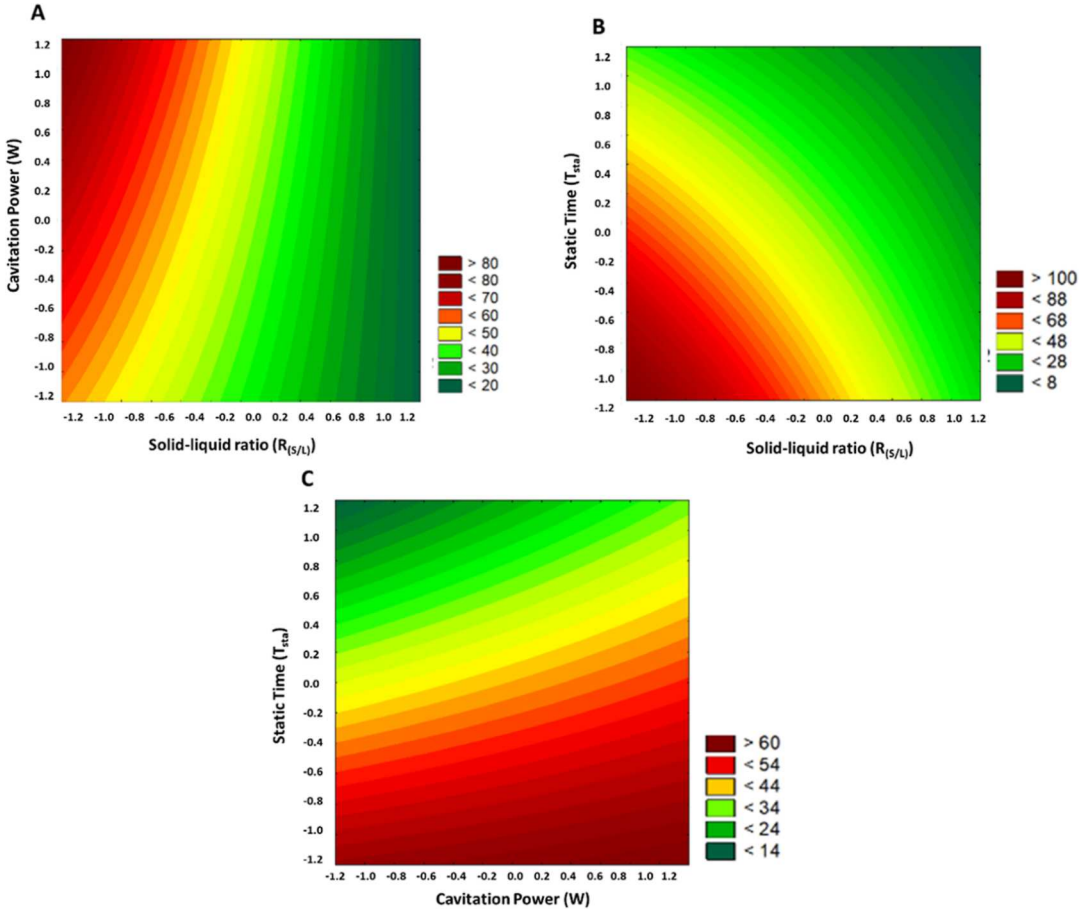

Figure 4. Response contour plots obtained from the CCRD predictive model (2^3 + axial points) for the yield of extraction of phenolic compounds from green propolis, considering ($R_{(S/L)}$), t_{sta} (min), and cavitation power (W) as independent variables. A combination of independent variables was performed 2 by 2 as follows: cavitation power (W) vs $R_{(S/L)}$ (A); t_{sta} vs $R_{(S/L)}$ (B); t_{sta} vs cavitation power (W) (C).

solvent is natural, inexpensive, non-toxic, and can be kept in the final product as a potent biological additive.

Operational Condition Optimization. *Ultrasonication-Assisted Extraction.* A step-by-step strategy was used to determine the best molar ratio of Bet/CA, which would result in the highest extraction yield ($mg_{polyphenol}$ $g_{propolis}^{-1}$) to optimize the solvent composition. Different molar ratios were tested (3:1, 2:1, 1:1, 1:2, and 1:3), all with 20 wt % of water and with the $R_{(S/L)}$ = 0.015. We chose a low water concentration to determine which solvent would perform best regardless of the presence of water. The initial test (Figure 3A), with a molar ratio of 1:2, resulted in the highest extraction

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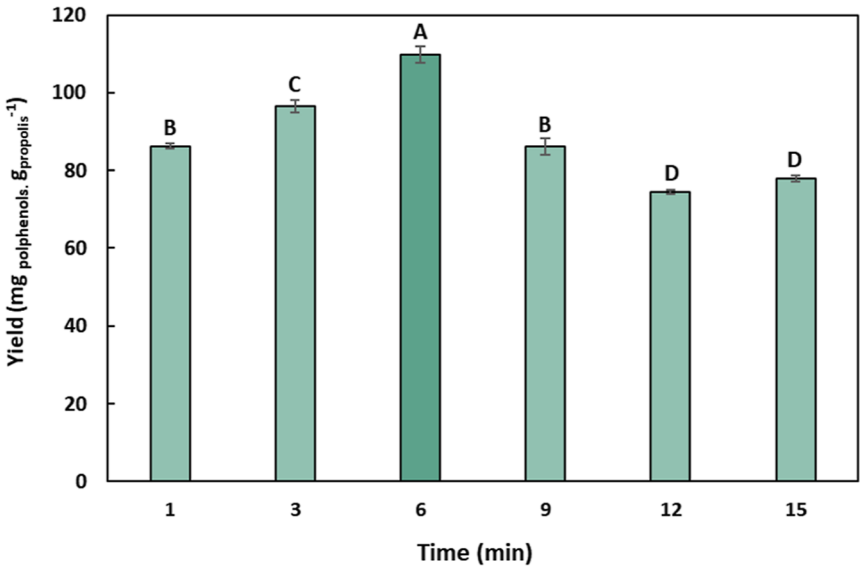

Figure 5. Data obtained for the yield of extraction of phenolic compounds from green propolis using different extraction times (1, 3, 6, 9, 12, and 15 min) but fixed conditions of ($R_{(S/L)}$ = 0.02, cavitation power = 400 W, and t_{sta} = 5 min). Different letters demonstrate statistically different results (p-value < 0.05 from ANOVA).

yield of 150 ± 3 $mg_{polyphenol}$ $g_{propolis}^{-1}$. Then, different percentages of water (20, 25, 30, 35, 40, 50, and 60%) were investigated while maintaining the molar ratio of 1:2, and the initial $R_{(S/L)}$ of 0.015. The best percentage was 50 wt % of water, which promoted the highest extraction yield of 165 ± 2 $mg_{polyphenol}$ $g_{propolis}^{-1}$ (ANOVA p < 0.05)—Figure 3B.

The combination of starting materials and water maximized the extraction performance. In addition, the artepillin C and p-coumaric acid molecules showed a similar trend in their sigma profiles, with one peak in the HBD (σ < −0.01 e/Å^2) and another in the HBA area (σ > +0.01 e/Å^2) related to the contribution of H atoms and hydroxyl groups of the leaves, respectively (Figure S3—Supporting Information). There was an intense peak in the non-polar area (−0.01 e/Å^2 < σ < 0.0084 e/Å^2) due to the contribution of the molecule's carbonic chain. Similarly, selected HBAs and HBDs have a large polar region, which makes them able to solubilize phenolic compounds efficiently.

Ultrasonic Probe. To maximize the extraction yield and reduce the screening time, we have replaced the ultrasonic bath extraction method (135 W) with an ultrasonic probe, with an operating capacity between 200 and 800 W. This mode of operation is expected to promote a more intense release of biomass compounds and temperature increase, as reported in the literature.[41,42]

However, it is essential to study the cavitation power carefully. For propolis samples, temperatures should not exceed 100 °C to prevent the degradation of flavonoids, which can occur at temperatures higher than 110 °C.[43] Thus, a short extraction time of 3 min was maintained initially. Furthermore, exploring a shorter extraction not only saves energy[30] but also allows for higher production by batch without compromising the extraction yield. To further optimize the extraction process, Bet/CA (1:2) with 50 wt % of water was used to compose the experimental design (Central Composite Rotatable Design—CCRD 2^3). The experimental plan was based on three operational variables: $R_{(S/L)}$, cavitation power (W), and static time (t_{sta}), the latest representing the contact between green propolis and eutectic solvent before extraction. The responsive variable used as a model to guide the optimization of the process was the total content of phenolic compounds ($mg_{polyphenol}$ $g_{propolis}^{-1}$). Table S3 (Supporting Information) presents each trial's planning details and corresponding results ($mg_{polyphenol}$ $g_{propolis}^{-1}$).

The extraction yield of total phenolic compounds from green propolis served as the dependent variable for the predictive model presented in eq 3. According to this equation, all tested variables were statistically significant and contributed to the predictive model with a 95% confidence level (p-value < 0.05). The Pareto chart (Figure S4—Supporting Information) revealed that the quadratic levels of the variables had a significant impact on the model, with $R_{(S/L)}$ and t_{sta} being the most relevant parameters. The coefficient of variation of the CCRD calculated using the central levels (tests 9−11) was around 14%. Furthermore, with an R^2 of 0.85, the model designed was considered to have a high predictive capacity.

$$y\ (\mathrm{mg/g}) = 44.16 - 20.98 \times (R_{SL}) + 5.289 \times (P) - 17.77 \times (t_{sta}) - 4.56 \times (R_{SL} \times P) + 6.91 \times (R_{SL} \times t_{sta}) + 2.36(P \times t_{sta}) \quad (3)$$

Figure 4 shows the predictive contours drawn using the model. Trial 3 ($R_{(S/L)}$ 0.02, 400 W, and t_{sta} of 5 min) resulted in the maximum phenolic extraction yield (100.38 (±2.53) $mg_{polyphenol}$ $g_{propolis}^{-1}$). In contrast, the lowest extraction yield (18.20 ± 2.09 $mg_{polyphenol}$ $g_{propolis}^{-1}$) was obtained in trial 6, which represents the highest $R_{(S/L)}$ (0.04), lower cavitation power (240 W), and longer t_{sta} (15 min). The responsive surface graphs (Figure 4) interpretation suggests that the optimal extraction of phenolic compounds from propolis is achieved with a smaller $R_{(S/L)}$ and lower t_{sta}. The cavitation power was found to have only minor interference with the extraction yield. The waxy nature of green propolis is responsible for the positive impact of smaller $R_{(S/L)}$ and lower t_{sta} on the extraction yield. This characteristic causes propolis to agglomerate, reducing its contact surface with the solvent and hindering the extraction performance. Three independent extractions were performed under ideal con-

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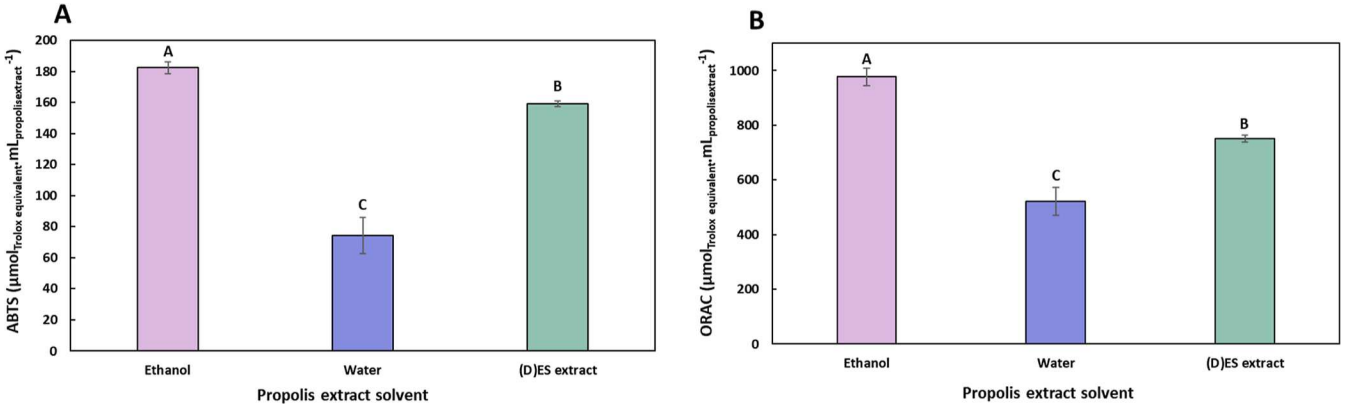

Figure 6. Antioxidant analysis: (A) ABTS ($\mu mol_{TroloxEquivalent}$ $mL_{propolisextract}^{-1}$) $n = 3$ for each group; (B) ORAC μmol $_{TroloxEquivalent}/mL_{propolisextract}^{-1}$ $n = 2$ for each group. All analyses were performed by fixing the optimal conditions of $R_{(S/L)} = 0.02$, ultrasonic probe (time of 6 min), with cavitation power of 400 W, t_{sta} of 5 min. The bars illustrate the results with the respective standard deviations. Different letters represent results that are statistically different (p-value < 0.05—ANOVA).

ditions, which included an $R_{(S/L)}$ of 0.02, cavitation power of 400 W, and a t_{sta} of 5 min, to validate the model. The accuracy and precision of eq 3 were verified by comparing the experimental results with predicted theoretical data. The model was validated with a relative deviation of 6.13%, indicating a high confidence level in the design model. This validation is presented in Table S4 in the Supporting Information, and the predictive vs experimental results are shown in Figure S5 in the Supporting Information.

The extraction time was optimized using the conditions $R_{(S/L)}$ of 0.02, cavitation power of 400 W, and t_{sta} of 5 min. Six different extraction times (1, 3, 6, 9, 12, and 15 min) were tested, and the performance of the extracts ($mg_{polyphenol}$ $g_{propolis}^{-1}$) was compared in Figure 5. The results denote that the optimal extraction was achieved at 6 min, which resulted in the highest yield (110 ± 2 $mg_{polyphenol}$ $g_{propolis}^{-1}$). Interestingly, the longer extraction times did not result in higher extraction yields, probably due to the temperature increase during the step, which starts to affect the phenolic compounds considering their thermosensitivity, as demonstrated in data of Table S5 in the Supporting Information.

To further improve our extraction method, various techniques under optimized conditions, including an ultrasonic bath, magnetic stirrer, and shaker, were evaluated to compare their extraction performance to that of the ultrasonic probe. As shown in Figure S6 (Supporting Information), the ultrasonic probe method, using Bet/CA as the solvent, proved to be the most efficient among the extraction approaches (expressed in $100\%_{relative}$ extraction yield). It is important to note that using the optimized final conditions is crucial. The ultrasonic bath, for example, only recovered $31\%_{relative}$ of the polyphenols when compared to the probe, reinforcing the need for the ultrasonic probe to accelerate the extraction of phenolic compounds from propolis and to produce more by batch without wasting energy and time, which means a more economical process.

Characterization of the Final Extract. Using the optimized conditions of ultrasonic probe extraction ($R_{(S/L)}$ of 0.02, power cavitation of 400 W, and t_{sta} of 5 min), during 6 min of cavitation, we compared the eutectic solvent with the conventional solvents, ethanol, and water. In Figure S7 (Supporting Information), ethanol still presented higher extraction yields than other solvents (135 ± 3 $mg_{polyphenols}$ $g_{propolis}^{-1}$). However, the DES-based extract yielded (104 ± 1 $mg_{polyphenols}$ $g_{propolis}^{-1}$) about 25 times higher than that obtained with water (5 ± 1 $mg_{polyphenols}$ $g_{propolis}^{-1}$). This aspect solves the problem of finding an alternative solvent to water that is also natural and non-toxic. Moreover, the resulting product from our platform was an extract that combined DESs with the phenolic compounds of propolis, eliminating the need for energy-intensive and time-consuming removal of the alternative solvent from the final formulation.[28]

Therefore, it is interesting to investigate whether the eutectic solvent interferes with the biological activities of the phenolic compounds after extraction. It is also necessary to assess the possibility of improving the benefits of the final product by maintaining the eutectic solvent in the extract obtained, which is rich in phenolic compounds. In this context, the antioxidant, antihypertensive, lipase inhibition, and antimicrobial potential of the extract rich in phenolic compounds obtained with the eutectic solvent was studied and compared with the conventional, aqueous, and ethanolic extracts.

Antioxidant Analysis. It is well known in the literature that phenolic compounds from propolis display antioxidant activity. However, due to the complexity of the green propolis matrix and the presence of many different types of phenolic compounds, this activity cannot be evaluated by a single method.[44] Therefore, we applied the ABTS radical scavenging activity test and ORAC in this work. ABTS is the most popular colorimetric method that applies to hydrophilic and lipophilic antioxidants, while ORAC is a more sensitive method to measure the activity of hydrophilic antioxidants.[45]

The results of the antioxidant analysis are presented in Figure 6. As expected, the ethanol extract provided the best results in both antioxidant analyses (ABTS, 182 ± 4 $\mu mol_{TroloxEquivalent}$ $mL_{propolisextract}^{-1}$; ORAC, 977 ± 31 $\mu mol_{TroloxEquivalent}$ $mL_{propolisextract}^{-1}$), thanks to the extraction of a more significant number of phenolic compounds.

As shown in Figure 6, the DES-based extract presents a high antioxidant activity (ABTS, 159 ± 2 $\mu mol_{TroloxEquivalent}$ $mL_{propolisextract}^{-1}$; ORAC, 750 ± 12 $\mu mol_{TroloxEquivalent}$ $mL_{propolisextract}^{-1}$), which is superior to water (ABTS, 74 ± 12 $\mu mol_{TroloxEquivalent}$ $mL_{propolisextract}^{-1}$; ORAC, 521 ± 50 $\mu mol_{TroloxEquivalent}$ $mL_{propolisextract}^{-1}$). Although this result may seem insignificant, it is critical because it demonstrates that the eutectic solvent does not interfere with the activity of phenolic compounds. Thus, removing the solvent from the final formulation is unnecessary if the goal is to apply it for antioxidant purposes.

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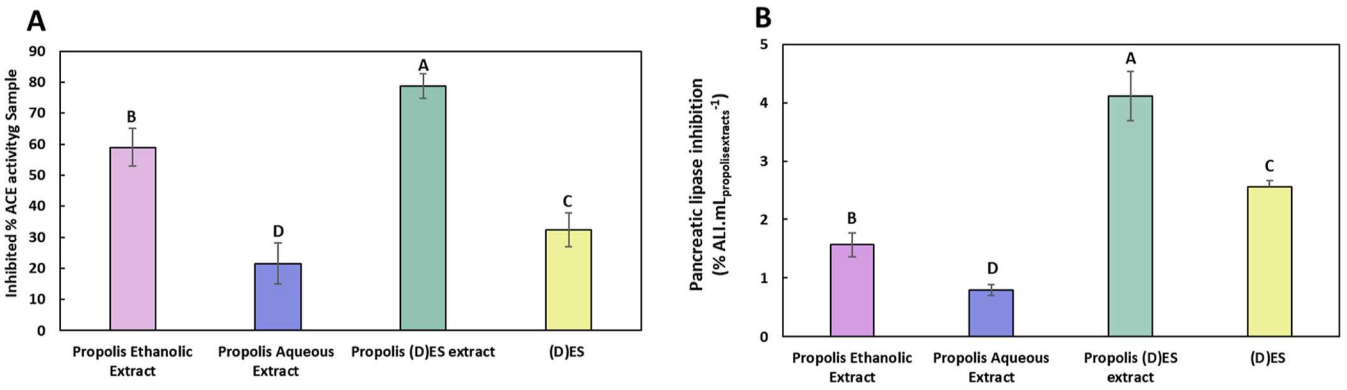

Figure 7. Green propolis biological analysis. (A) % of inhibition of angiotensin I to convert the ACE enzyme; $n = 2$ for each group. (B) Pancreatic lipase inhibition assay; % lipase activity inhibited per mL of propolis extract (% ALI $mL_{propolisextracts}^{-1}$); $n = 2$ for each group. Parameters fixed in all analyses: $R_{(S/L)}$ of 0.02, ultrasonic probe (time of 6 min), with a cavitation power of 400 W, and a t_{sta} of 5 min. The bars illustrate the results with respective standard deviations. Different letters represent statistically different results (p-value < 0.05—ANOVA).

ACE-Inhibitory Activity Assay and Pancreatic Lipase Inhibition. The extracts' antihypertensive and lipase inhibition activities were assessed to evaluate the biological effects of propolis extracts. Additionally, the isolated effect of the eutectic solvent, without phenolic compounds from propolis, was also tested for both analyses to determine whether there were any synergistic effects between the solvent and the polyphenols extracted. The ACE inhibitory activity of propolis samples (0.02 g mL^{-1}), expressed as a percentage of inhibition of angiotensin *I* converting enzyme (ACE), is shown in Figure 7A.

Figure 7 reveals that all extracts, including ethanolic (59 ± 6 % ACE inhibition), aqueous (22 ± 6% ACE inhibition), and the extract with the eutectic solvent (79 ± 4% ACE inhibition), exhibited antihypertensive activity. According to the literature, ethanolic extracts of green propolis may help prevent hypertension, with the effects being positively correlated with flavonoid compounds.[46] However, our data demonstrate that the ethanolic extract, with the highest phenolic compound content, did not present the highest antihypertensive activity. Instead, the highest percentage of antihypertensive activity was achieved with the propolis extract using the eutectic solvent, which can be explained by the isolated solvent also exhibiting a percentage of inhibition (32 ± 4% ACE inhibition).

The same behavior was observed for the pancreatic lipase inhibition assay. Figure 7B shows that all extracts—ethanolic (1.5 ± 0.2% ALI $mL_{propolisextracts}^{-1}$), aqueous (0.79 ± 0.08% ALI $mL_{propolisextracts}^{-1}$), and the one with the eutectic solvent (4.1 ± 0.4% ALI $mL_{propolisextracts}^{-1}$) have demonstrated anti-lipase activity, which was briefly demonstrated already in the literature.[47]

The anti-lipidic capacity of propolis may be attributed to the presence of terpenes/monoterpenes.[48] However, there is still a lack of answers regarding the mechanisms involved, as evidenced by in vitro studies. Despite this characteristic of propolis components, the highest percentage of inhibition was achieved with the propolis extract made using the eutectic solvent. This aspect could be explained by the fact that the isolated solvent also exhibited a percentage of inhibition (2.6 ± 0.1% ALI $mL_{propolisextracts}^{-1}$).

Both antihypertensive and anti-lipidic effects can be further elucidated by studying the composition of the solvent. Betaine, known for its osmotic properties and antioxidant and anti-inflammatory effects, has been shown to protect against alcohol-induced hepatic steatosis, apoptosis, and the accumulation of damaged proteins.[49] Thus, it can be inferred that betaine plays a critical role in several biological mechanisms.

Based on these findings, it can be concluded that the propolis DES-based extract has the potential to be a formulation for biochemical studies aimed at designing dietary therapies to control chronic diseases. There is enormous potential associated with positive results in managing microvascular complications exhibited by the extract, as it possesses antioxidant and macrovascular activities by inhibiting the angiotensin I converter and the pancreatic lipase. This aspect is particularly important, given the growing emphasis on natural formulations that combat chronic diseases such as diabetes, obesity, cardiovascular diseases, and cancer in modern nutritional science.[50]

Furthermore, there is an urgent need for natural formulations to replace synthetic drugs. For instance, the lipase inhibition mechanisms are the most widely studied pathway for identifying potential anti-obesity agents. Despite the many plant and microbial origin molecules studied for their pancreatic lipase inhibition, only one blockbuster drug, orlistat, has been approved by the FDA.[51] Therefore, discovering natural extracts with anti-obesity activity, such as the propolis DES-based extract, is a promising development for managing chronic diseases.

With the recent COVID-19 pandemic, propolis has been investigated as a potential auxiliary treatment for patients. According to the World Health Organization (WHO), propolis can be combined with other drugs without the risk of inactivating the conventional COVID-19 treatment.[52−54] Although the exact mechanism is not yet clear, our results suggest that the presence of the solvent in the final extract positively impacts the beneficial activities of propolis. Therefore, enhancing the observed effects of propolis could be a promising strategy against inflammatory diseases since the biological effects of the propolis components are combined with those of the eutectic solvent.

Bacterial Growth Inhibition. In addition to diseases, infections are a significant contributor to global health loss. This trend has been aggravated by the increase in antimicrobial resistance, in which certain bacteria survive the drugs that usually eliminate or impede their growth.[50] Furthermore, the likelihood of epidemic events such as the COVID-19 pandemic

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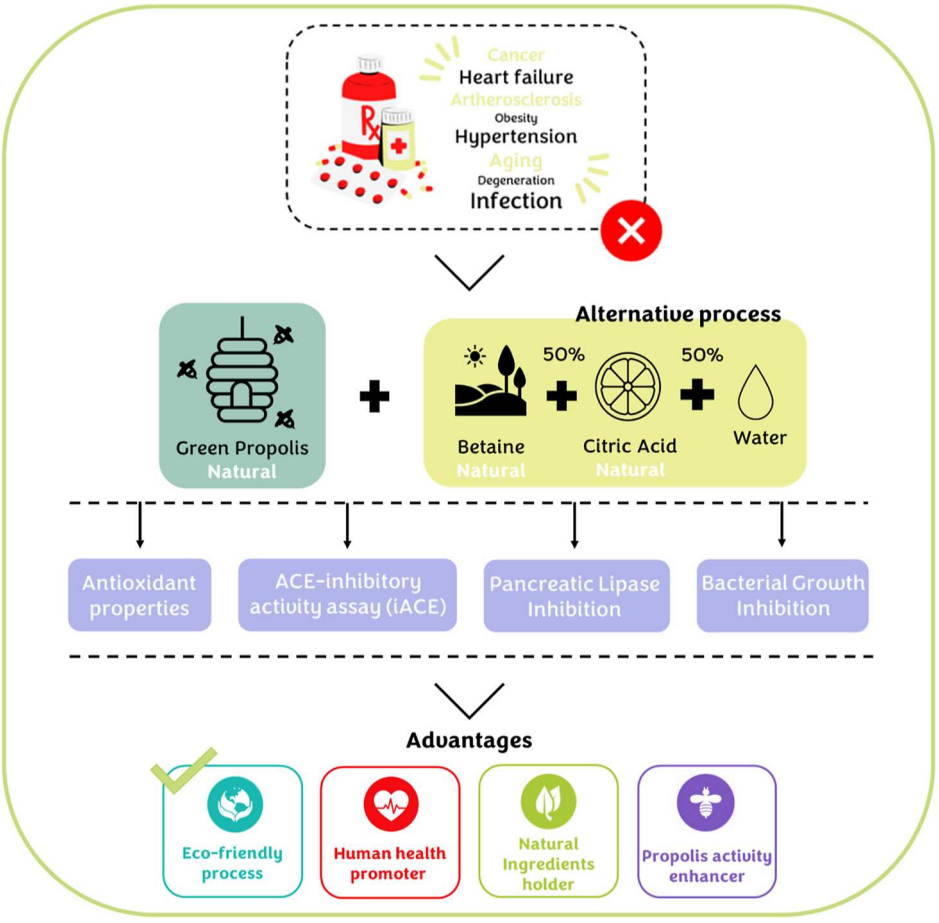

Figure 8. Summary of the biological potential and mechanisms of the DES-based extract of propolis.

is rising.[52] As a result, the inevitable threat of infections makes it necessary to develop alternative therapies, either preventive or adjuvant, to combat them.

It is widely known that both ethanolic and aqueous propolis extracts possess antimicrobial properties, exhibiting activity against bacteria, fungi, parasites, and viruses.[55,56] Over 600 bacterial strains, including those resistant to conventional antimicrobial agents, have been tested for propolis's antimicrobial potential. The results show that most bacterial strains, including *E. coli*, *S. aureus*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Enterococcus* spp., *Yersinia enterocolitica*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Streptococcus mutans*, *S. epidermidis*, *Aeromonas* spp., *Mycobacterium tuberculosis*, and *Bacillus cereus*[57−60] are susceptible to propolis. Although there has been less research conducted on the antifungal, antiparasitic, and antiviral effects of propolis in comparison to its antibacterial properties, the findings seem quite promising.

Thus, it is observed that the literature contains a significant amount of information on the antimicrobial properties of conventional propolis extracts. Therefore, our study aimed to determine the concentration of propolis extract that would result in a 50% inhibition of bacterial growth after 24 h (IC_{50}), using the IC_{50} of the propolis extract made with the eutectic solvent as a reference point. Then, the same concentration of the ethanolic and aqueous extracts was assessed to assess if similar effects could be achieved using different solvents. The IC_{50} ($g_{propolis}$ mL^{-1}) of the eutectic solvent extract is presented in Table S6 in the Supporting Information, and the percentage of inhibition achieved by applying this IC_{50} to the other solvents and the bacterial growth inhibition obtained using this IC_{50} during the 24 h is illustrated in Figure S8 in the Supporting Information.

Our results demonstrate that the extract with the eutectic solvent could not inhibit at least 50% of the bacterial growth in other extracts when tested against the set of microorganisms under study in this work. The pH of the pure eutectic solvent (pH = 2) and of the propolis extract with the eutectic solvent (pH = 3) may contribute to this limitation, as compared to the neutral solvent (pH = 7) of the ethanolic extracts resuspended with DMSO and the aqueous extract. Nonetheless, a synergistic effect was observed when the eutectic solvent was combined with propolis against *S. aureus* and *C. albicans*, both Gram-positive bacteria. This result is consistent with previous studies indicating that green propolis is more efficient against Gram-positive bacteria.[58,61] These preliminary results indicate that the eutectic solvent may have a significant potential for combating microorganisms, including multi-resistant strains. However, further assays are required to investigate the biological potentials and mechanisms of action of this eutectic solvent extract on bacteria, despite the hypothesis raised about pH.

Environmental Analysis—EcoScale. The potential biological mechanisms of the DES-based extract of propolis are summarized in Figure 8. The figure highlights the importance of the formulation, which is derived from a combination of propolis, a natural product, and two other natural substances. Citric acid, found in most fruits, especially citrus fruits like

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lemon and orange, is one of the substances,[62] while betaine, a widely distributed substance in animals, plants, and microorganisms, is the other.[49] This formulation is relevant because natural products contribute to 35% of substances used in modern medicine, with 25% being attributed to plants and 13% to microorganisms. However, in many cases, the solvents used to extract such substances can be harmful and toxic to humans.[63]

In this study, we used the EcoScale tool to evaluate the environmental impact of the optimized ultrasonic probe extraction compared to other extraction techniques, including maceration, magnetic stirring, shaker, and ultrasonic bath. The amount of product obtained was analyzed and measured in $mg_{polyphenol}\ g_{propolis}^{-1}$ expressed in $\%_{relative}$. The results were presented in Table S7 of the Supporting Information, which includes penalty points, consequently ranging an EcoScale score from 0 to 100, with higher scores indicating better environmental sustainability.

All the techniques were penalized with three points in the price parameter due to the use of Bet/CA, a safe solvent that is not conventionally used on a large scale, limiting its industrial application. Additionally, the ultrasonic probe and ultrasonic bath suffered 5 penalty points (3 points for the technical setup and 2 points for the temperature) due to their high energy use and heat dissipation. Magnetic stirring received a penalty of 1 point. Despite the penalties imposed on the ultrasonic probe-optimized extraction technique, it achieved the highest score of 92. Therefore, the extracted product is considered a greener and more sustainable alternative, as confirmed by the EcoScale analysis, and promotes human well-being, as its biological effects have been demonstrated.

CONCLUSIONS

This study successfully developed a new extraction process for Brazilian green propolis using a eutectic solvent-based approach. Among the various solvents tested, Bet/CA (1:2) with 50 wt % of water (v/v) was the most efficient in selectively extracting propolis polyphenols, with a maximum extraction yield of 100 $mg_{polyphenol}\ g_{propolis}^{-1}$ at the optimum conditions (ultrasonic probe at 400 W, t_{sta} of 5 min for 6 min). The resulting extract had a high biological value and outperformed water as a solvent. The presence of the eutectic solvent in the extract was also found to have a positive impact on biological assays, indicating that this eutectic solvent could be used as a bioactive additive in the final extract. This process is straightforward and can be performed using widely used techniques, making it an innovative and promising method for creating an extract with antioxidant, antihypertensive, antilipase, and antimicrobial properties. The propolis eutectic solvent extract is both natural and non-toxic, making it an excellent option for future food, cosmetic, and pharmaceutical applications. However, further studies are needed to determine the extract's efficiency in vivo and its effects on people and microorganisms. Overall, the reported process is a significant step forward in developing a high-quality propolis extract with numerous potential applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.3c03812.

Additional information about COSMO-RS, CCRD experimental, extraction efficiency comparing different techniques/solvents, and bacterial growth inhibition details (PDF)

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Notes

The authors declare no competing financial interest.

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