Supporting Information

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

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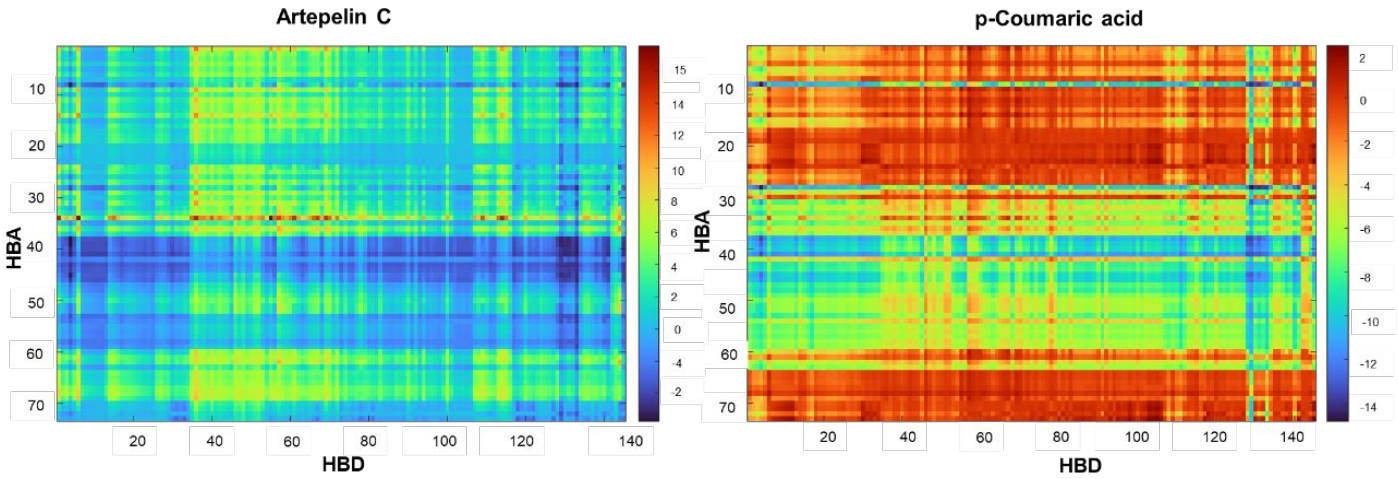

Figure S1. The activity coefficients at infinite dilution (lnγ∞) of of Artepelin C (left) and p-cumaric acid (right) in different deep eutectic solvents (1:1) at 308.15 K.

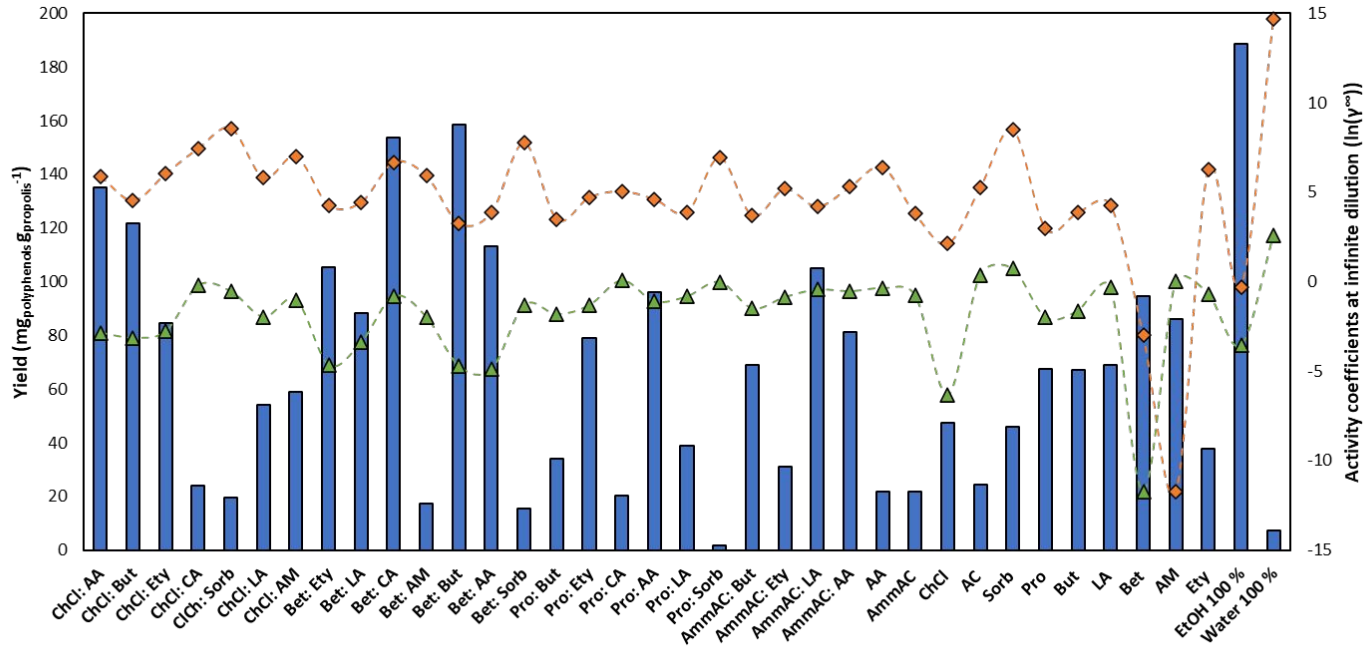

Figure S2. Yield of extraction of phenolic compounds from propolis with various eutectic solvents and their isolated starting materials *versus* the activity coefficients at infinite dilution (lnγ∞) of Artepillin C (in orange) and p-Coumaric acid (in green).

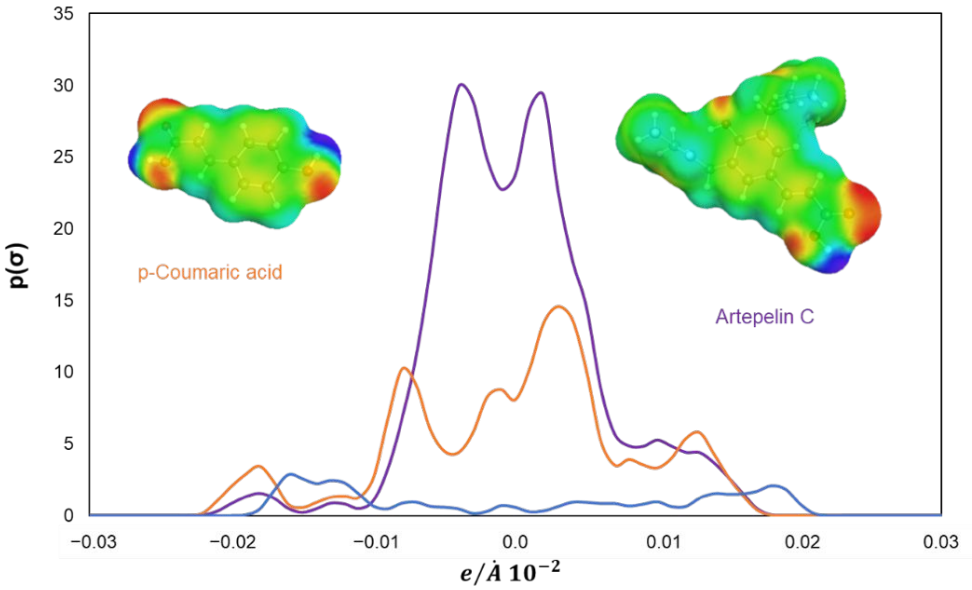

Figure S3. The σ-profiles (COSMO-RS) of artepillin C (in purple), *p*-coumaric acid (in organge), water (in blue) and 3D induced surface charge densities.

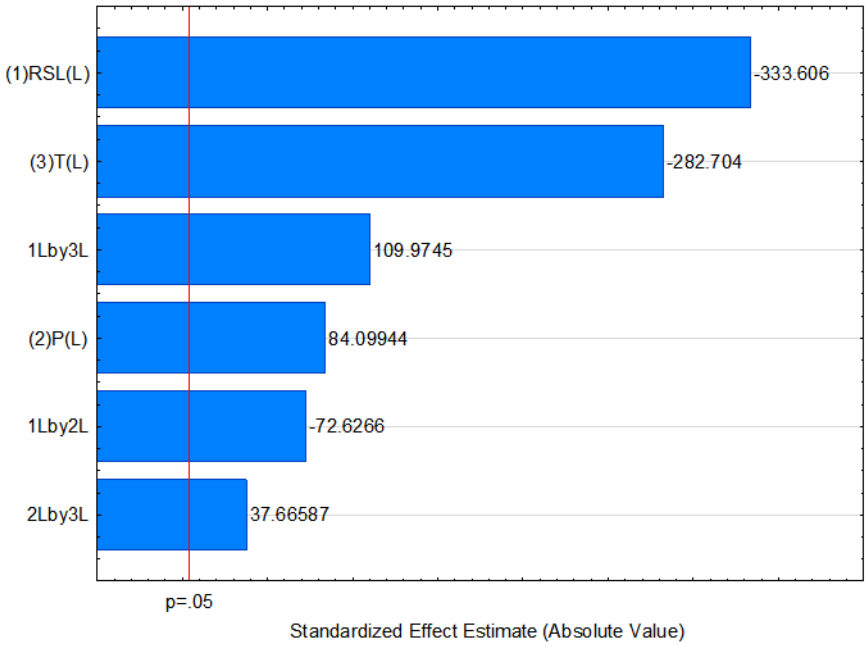

Figure S4. CCRD Pareto´s chart (2^3 + central points) obtained for the extraction of phenolic compounds with Bet:CA, 1:2 with 50 wt% of water) during 3 minutes.

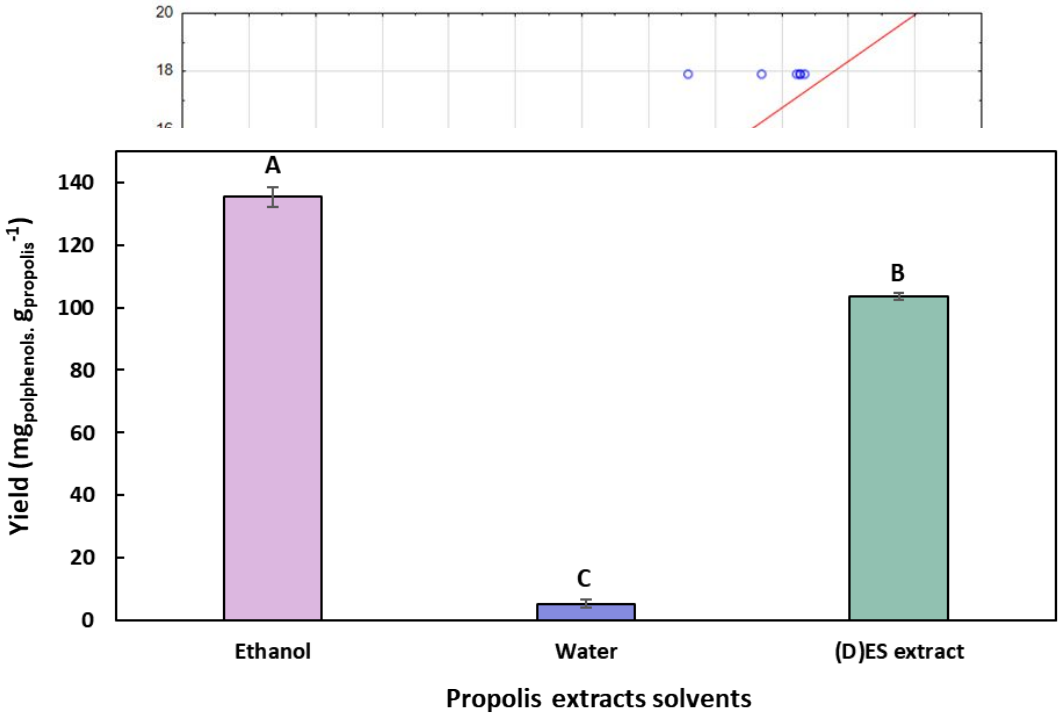

Figure S5. Predicted vs. experimental results obtained from CCRD studies (2^3 + central points) regarding the extraction yield of phenolic compounds from propolis using Bet:CA, 1:2).

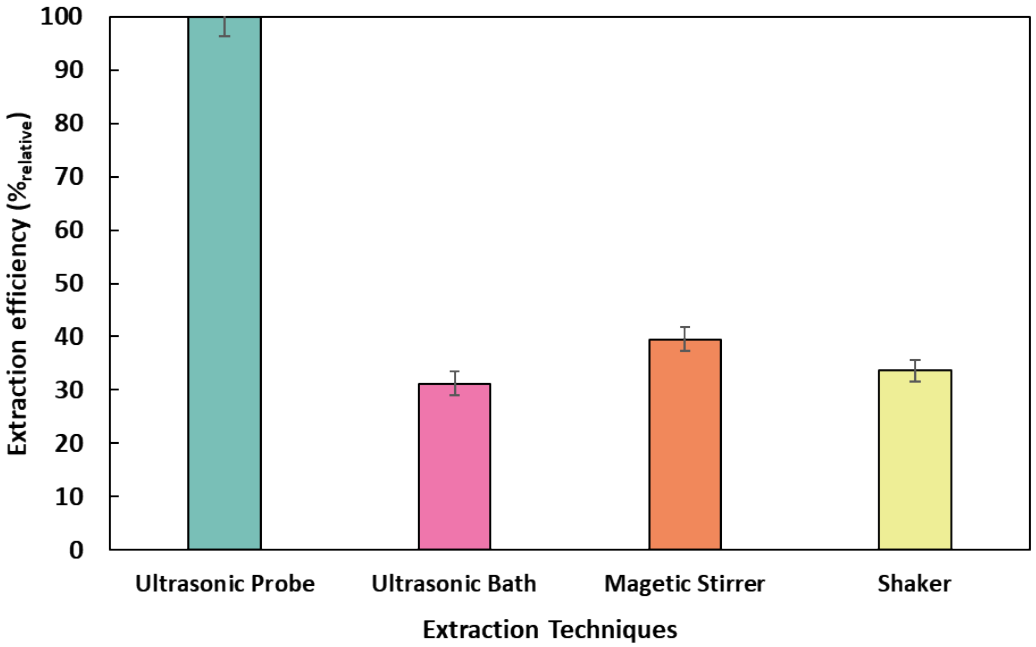

Figure S6. Comparison of the recovery (%relative) of propolis phenolic compounds obtained with different extraction methods (Ultrasonic probe, ultrasonic bath,magnetic stirrer, shaker) using the optimized conditions Bet:CA, $R_{(SL)}$ = 0.02, t_{sta} of 5 min and 6 min of extraction.

Figure S7. Extraction yield of phenolic compounds from propolis by applying Ultrasonic Probe extraction ($R_{(S/L)}$ = 0.02, cavitation power of 400 W and t_{sta} of 5 min), for 6 minutes of extraction time, for the eutectic solvent selected, and the conventional solvents ethanol and water. The bars show the extraction yield in $mg_{polyphenol}. g_{propolis}^{-1}$ with the standard deviation of the extraction done in triplicate. Different letters demonstrate results statistically different (*p*-value < 0.05 from ANOVA)

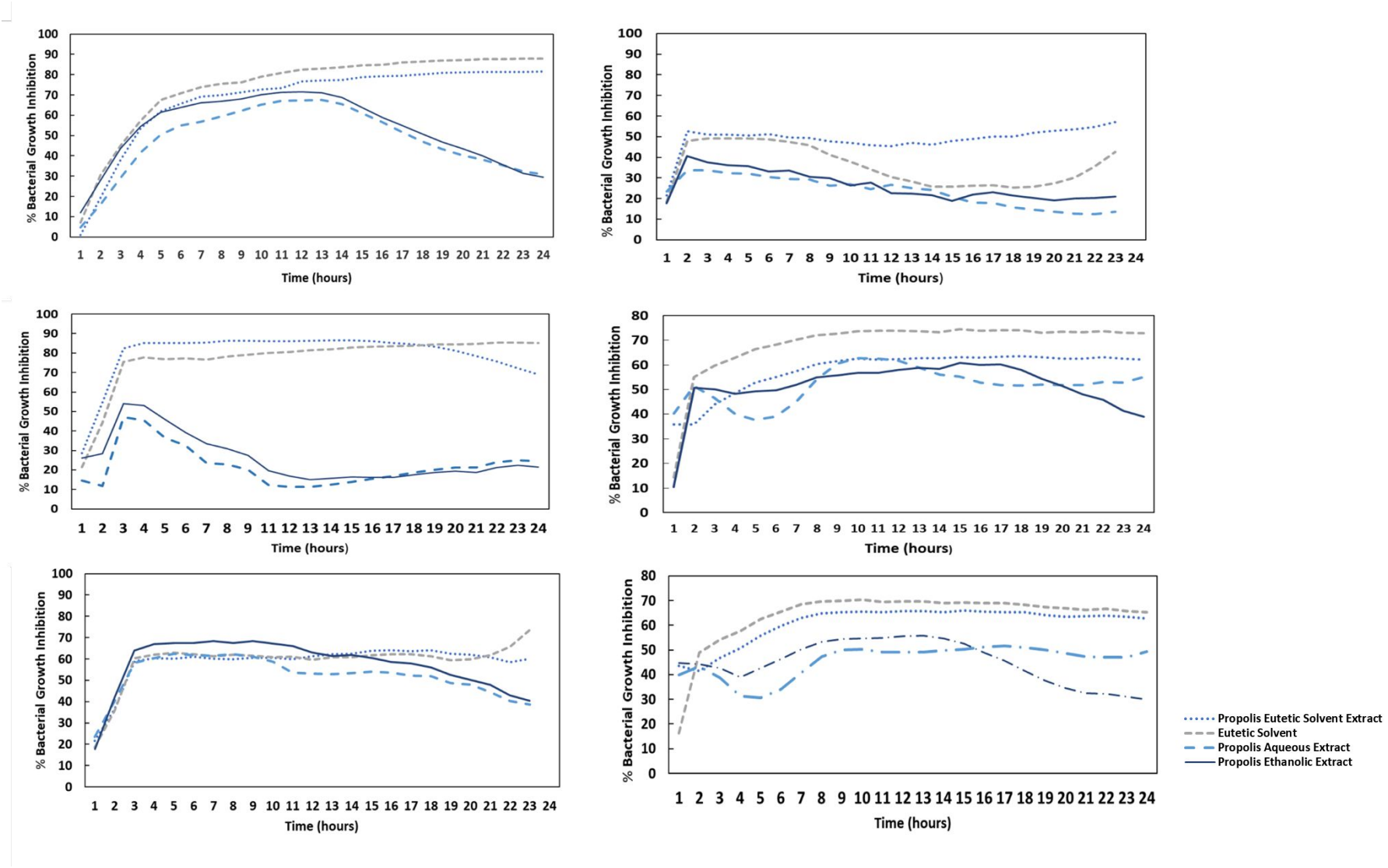

Figure S8. Growth curves of six microorganisms, namely *Staphylococcus aureus*, *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus epidermidis* and *Candida albicans* by appling IC50.

Tables

Table S1. List of HBAs used in the COSMO-RS screening.

Code	HBA	Code	HBA
1	Urea	37	Diethyl ethanol ammonium Chloride
2	Acetamide	38	Tetraethylammonium chloride
3	Nicotinamide	39	Tetraethylammonium chloride
4	Threonine	40	Tetrapropylammonium chloride
5	Ornithine	41	Tetrabutylammonium chloride
6	Citrulline	42	Tetraheptyl ammonium chloride
7	Trimethylglycine	43	Tetraoctyl ammonium chloride
8	Betaine	44	Methyl trioctyl ammonium chloride
9	Glycine	45	Triethylmethylammonium chloride
10	Proline	46	Benzyltriethylammonium chloride
11	Serine	47	benzyl_trimethyl ammonium chloride
12	Alanine	48	Phenyltrimethylammonium chloride
13	Histidine	49	N-ethyl-2-hydroxy-N.N-dimethylethanaminium chloride
14	Thereonine	50	2-(chlorocarbonyloxy)-N.N.N-trimethylethanaminium chloride
15	Lysine	51	Tetramethylammonium bromide
16	Arginine	52	Tetramethylammonium_bromide
17	Lactic acid	53	Tetrapropylammonium bromide
18	Malic acid	54	Tetrabutylammonium bromide
19	Citric acid.	55	Methyltriphenyl phosphonium bromide
20	Octanoic acid	56	Methyltriphenyl phosphonium bromide
21	Nonanoic acid	57	Benzyltriphenylphosphonium bromid
22	Decanoic acid	58	Methyl trioctyl ammonium bromide
23	Dodecanoic acid	59	Allyltriphenyl phosphonium bromide
24	Oxalic acid	60	Guanidine hydrochloride
25	Glycerol	61	Choline dihydrogen citrate
26	1-propanol	62	Choline dihydrogen phosphate
27	Ethylene glycol	63	Benzyl(2-hydroxyethyl)dimethylammonium chloride
28	Choline acetate	64	D-Glucose
29	Sodium acetate	65	L-Glucose
30	Ammonium acetate	66	Fructose
31	Sodium propionate	67	Sucrose
32	Cholinium chloride	68	Maltose
33	Choline bitartrate	69	Xylose
34	Ethylammonium chloride	70	Phenol
35	Acetylcholine Chloride	71	o-Cresol
36	Carnitine hydrochloride	72	Menthol
		73	Thymol

Table S2. List of HBDs used in the COSMO-RS screening.

Code	HBD	Code	HBD
1	Urea	44	L-Glucose
2	Dimethylurea	45	Isosorbide
3	N-methylurea	46	Maltose
4	N,N-Dimethylformamide	47	Mannose
5	Acetamide	48	Raffinose
6	Thiourea	49	Rhamnose
7	1-Hexanol	50	Sorbose
8	1-Octanol	51	Sucrose
9	1-Decanol	52	Trehalose
10	1-Dodecanol	53	Xylose
11	1-Tetradecanol	54	D-Ribose
12	1-Hexadecanol	55	Formic acid
13	Cyclohexanol	56	Acetic acid
14	Glycerol	57	Aconitic acid
15	Ethylene-glycol	58	Ascorbic acid
16	1,2-Propanediol	59	Citric acid
17	1,3-Butanediol	60	Fumaric acid
18	2,3-Butanediol	61	Glycolic acid
19	1,5-Pentanediol	62	Lactic acid
20	1,6-Hexanediol	63	Levulinic acid
21	1,7-Heptanediol	64	Maleic acid
22	1,8-Octanediol	65	Malic acid
23	1,9-Nonanediol	66	Malonic acid
24	1,10-Decanediol	67	Oxalic acid
25	1,15-Pentadecanediol	68	Porpionic acid
26	1,4-Butanediol	69	Succinic acid
27	Diethylene glycol	70	Tartaric acid
28	Triethyleneglycol	71	Mandelic acid
29	Furfuryl alcohol	72	Aspartic acid
30	2,4,6-Trimethyl phenol	73	Benzoic acid
31	2-Methyl-phenol	74	Salicylic acid
32	3,4-Dimethyl-phenol	75	Acetylsalicylic Acid
33	4-Chloro phenol	76	Phenylacetic acid
34	4-Methoxy phenol	77	p-Coumaric acid
35	Erythrito	78	Gallic Acid
36	Inositol	79	p-Hydroxy-benzoic acid
37	Mannitol	80	Nicotinic acid
38	Sorbitol	81	p-Toluenesulfonic acid
39	Xylitol	82	Phenylpropionic acid
40	Arabinose	83	Itaconic acid
41	Fructose	84	3-Phenyl-propionic acid
42	Galactose	85	4-Phenyl-butyric acid

43	S-Glucose	86	5-Phenyl-valeric acid
87	Butyric acid	124	p- Hydroxybenzyl-alcohol
88	Valeric acid	125	p-Hydroxy-benzaldehyde
89	Phenylacetic acid	126	Vanillin
90	Glutaric acid	127	o-Cresol
91	Leucine	128	Ethanolamine
92	Vanillic acid	129	Benzyltriethylammonium chloride
93	Geranic acid	130	Camphor
94	Pyruvic acid	131	Pyridine
95	Octanoicacid	132	Quinoline
96	Nonanoic acid	133	Trioctylphosphine oxide
97	Decanoic acid	134	Benzamide,
98	Dodecanoic acid	135	Catechol
99	Caproic acid	136	Hydroquinone
100	Glutamic acid	137	Paracetamo
101	Hexanoic acid	138	Pyrrole
102	Ricinoleic acid	139	Aspirin
103	Hexadecanoic acid	140	2-Methylimidazole
104	Tetradecanoicacid	141	Chimaphilin
105	Oleic acid	142	Dihydroquercetin
106	Lauric acid	143	Quercetin
107	Alanine	144	Zinc Chloride
108	Arginine	145	Ferric chloride
109	Glycine		
110	Histidine		
111	Lysine		
112	Proline		
113	Serine		
114	Thereonine		
115	Gulonolactone		
116	Fucose		
117	Menthol		
118	Thymol		
119	α-Naftol		
120	Resorcinol		
121	Phenol		
122	p-Chlorophenol		
123	Guaiacol		

Table S3. Predicted results compared with the experimental values obtained by the adjusted model and the relative percentage of the stable independent variables under the optimum conditions that guarantee the high yield of extraction of polyphenol compounds. V1, V2 and V3 represent the validation tests.

Assays	Solid Liquid $g_{propolis}.mL_{solvent}^{-1}$	Cavitation Power (W)	Static Time (t_{sta}) minutes	Experimental values	Predicted values	Error (%)
				Yield ($mg_{polyphenol}.g_{propolis}^{-1}$)		
V1	-1	1	-1	101.20	97.31	6.13
V2				105.85		
V3				103.97		

Operational variables			Response
$R_{(S/L)}$	Cavitation Power (W)	Static Time (t_{sta}) (minutes)	Polyphenol Extraction ($mg_{polyphenol}.g_{propolis}^{-1}$)
X1	**X2**	**X3**	**Y**
0.02 (-1)	240 (-1)	5 (-1)	91.33 (± 1.59)
0.04 (1)	240 (-1)	5 (-1)	38.75 (±1.25)
0.02 (-1)	400 (1)	5 (-1)	100.38 (± 2.53)
0.04 (1)	400 (1)	5 (-1)	41.37 (± 2.13)
0.02 (-1)	240 (-1)	15 (1)	31.28 (± 1.93)
0.04 (1)	240(-1)	15 (1)	18.20 (± 2.09)
0.02 (-1)	400 (1)	15 (1)	61.65 (± 0.53)
0.04 (1)	400 (1)	15 (1)	18.46 (± 3.10)
0.03 (-0)	320 (0)	10 (0)	21.83 (± 0.38)
0.03 (0)	320 (0)	10 (0)	28.32 (± 1.59)
0.03 (0)	320 (0)	10 (0)	28.03 (± 1.24)

Table S4. Real and encoded values of the optimization process expressed to guarantee yields of green propolis polyphenols by CCRD (2^3 + central points)

Table S5. Initial and final temperatures of the extraction system when applied different times of extraction (1, 3, 6, 9, 12 and 15 min).

Time (minutes)	1	3	6	9	12	15
$T_{initial}$ (°C)	36.7	36.5	36.4	36.3	36.6	36.0
T_{final} (°C)	60.2	78.7	84.7	90.4	92.7	90.8

Table S6. Microorganisms and related IC_{50} of eutetic solvent extract ($g_{propolis}.mL^{-1}$) and percentage (%) of inhibition applying this IC_{50}.

Microorganism (IC_{50})	Ethanolic Extract (%)	Aqueous Extract (%)	Eutetic Solvent Extract (%)	Eutetic Solvent (%)
Staphylococcus aureus (0.0033)	29.44	30.88	87.80	81.47
Escherichia coli (0.0028)	24.51	21.28	68.93	85.24
Methicillin-resistant *Staphylococcus aureus* (MRSA) (0.004)	38.97	45.03	62.63	72.80
Methicillin-sensitive *Staphylococcus aureus* (MSSA) (0.004)	29.95	44.14	62.61	65.25
Staphylococcus epidermidis (0.0033)	40.48	38.56	60.14	73.70

Candida albicans (0.0033)	21.04	13.50	57.15	42.47

Technique	Yield ($\%_{relative}$)	Price/availability	Safety	Technical setup	Temperature/time	Workup/Purification	EcoScale
Ultrasonic probe	100	-3	0	-3	-2	0	92
Ultrasonic Bath	31.18	-3	0	-3	-2	0	23.18
Magnetic stirring	39.56	-3	0	-1	0	0	35.56
Shaker	33.64	-3	0	0	0	0	30.64

Table S7. Penalty points regarding the environmental analysis done by the Ecoscale for each extraction technique and solvent used in the extraction of propolis polyphenols.