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ORIGINAL ARTICLE

COSMO-SAC model approach for deep eutectic solvent selection to extract quercetin from macela (*A. satureioides*) and experimental process optimization

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Abstract
In this work, deep eutectic solvents (DES) were evaluated like food-grade solvents, combined with an ultrasound-assisted technique for the extraction of quercetin from macela (*Achyrocline satureioides*). A COSMO-SAC (COSMO—segment activity coefficient) tool was applied to assist the solvent choice. From all evaluated DES, the composition based on choline chloride:glycerol:water (CGH2) was considered the most suitable to extract quercetin and other phenolics. The optimized process conditions by face-centered cubic design were the following: 90 min, 55 °C, and 1:100 solid-solvent ratio. According to chromatographic analysis, CGH2 showed high quercetin (27.7 mg/100 g) and catechin (26.1 mg/100 g) extraction capability. The higher quercetin content reinforces the ability of the predictive tools to support the solvent choice. Additionally, phenolic acids, *p*-coumaric, and Rosmaniric were reported for the first time in the *A. satureioides* extracts. Finally, was proposed a green and efficiency method to recovery quercetin and other phenolics from macela.

Keywords Green solvent · Predictive models · Ultrasound-assisted extraction · Face-centered cubic design · Bioactive compounds

1 Introduction

Medicinal plants are extensively used for years to treat diseases, and one of them is the *Achyrocline satureioides* (Lam.) D.C., commonly known as macela. This species has already been successfully applied in the treatment of gastrointestinal inflammation, respiratory problems, liver disorders, viral infections, and as anti-atherosclerotic and sedative agents [1]. These pharmacological effects are directly correlated to the presence of flavonoids, which are secondary metabolites with antioxidant activity [2].

The potential nutraceutical properties of these natural matrices induced the scientific community to develop new and sustainable processes to improve the interest compounds' recovery, and consequently, to value poor-explored plant materials. To achieve the current industry requirements, an ideal process needs to attend the green chemistry postulate; beyond present high biocompatibility with the target compounds; i.e., preserve the biological activity; and/or improve their nutraceutical quality [1]. In this context, the use of green solvents for extraction is an essential step in sustainable process development to obtain pharmaceuticals, food supplements, and other nutraceutical products from natural matrices [3–6].

The deep eutectic solvents (DES), is an ascending class of green solvents, extensively applied for extraction processes due to their distinctive properties which allow them to solubilize several classes of metabolites and, such as bioactive compounds, gluten, starch, DNA, proteins, and polysaccharides, even those poorly water-soluble [7–11]. DES is formed by hydrogen bonding interactions of a hydrogen bond acceptor (HBA), frequently ammonium halides, with a neutral hydrogen bond donor (HBD)

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species, such as amides, organic acids, sugar, or alcohols [7, 12]. When these components are mixed, their melting point is reduced considerably in comparison with the expected for the ideal solutions [13]. DES are also characterized by their low vapor pressure, non-flammability, non-volatility, high conductivity, thermal stability, high solvation capacity, and others [7, 12, 13]. To further a most properly solvent choice, or its components in the case of the DES, in silico tools, such as the COSMO-based models (conductor-like screening model), have been successfully applying. These theoretical models are able to describe the solute–solvent affinity considering the charges in the molecule configuration [14–16].

As the solvent, the employed extraction method is also a key factor in the developments of effective extraction processes [17]. In order to maximize the recovery of bioactive compounds, as well as simplify manipulation and work-up, ultrasound-assisted extraction (UAE) is widely used to reduce the extraction time [18], solvent consumption, and operation temperature [6, 19–21]. Nevertheless, the application of the UAE to obtain bioactive compounds requires process optimization related to the operational parameters, such as time, temperature, and solid-to-solvent ratio.

Taken the exposed into consideration, this work aimed to obtain a polyphenolic-enriched extract from *A. satureioides* using the DES combined with UAE. The DES were composed of choline chloride (ChCl) as HBA, and the effect of HBD of different classes was evaluated (alcohols, sugars, and acid). The in silico tool COSMO-SAC (COSMO—segment activity coefficient) was applied to assist the solvent choice. The outstanding process conditions were determined by the antioxidant activity of the extracts through different in vitro assays. For that, response surface methodology (RMS) was applied to determine the variable's effects and their interactions on the response variables. The phenolic profile concerning the main components was determined by chromatography (HPLC).

2 Material and methods

2.1 Chemicals

The chemicals used in this work are described in the Supplementary Material.

2.2 Preparation and characterization of Achyrocline satureioides

The preparation of the *A. satureoides* raw material was carried out as described by [34]. The dry material was donated by *Chamel Indústria e Com. de Produtos Naturais* (Campo Largo, Brazil). The approximate composition of the macela powder is shown in the Supplementary Material.

2.3 DES preparation

The evaluated DES were selected according to the affinity with quercetin, the major phenolic compound found in macela, taken into consideration that data provides by the current literature as described in Table 1. The DES preparation was carried out heating (50 °C) the HBA-HBD mixtures at an appropriate mixing molar ratio in a water bath with agitation (Dubnoff Orbital 304-TPA, Ethik Technology, Brazil).

2.4 Extraction procedure

The 37.5 mg of macela sample and 1.5 mL of each DES were vigorously stirred in 2-mL Eppendorf® tubes (solid-to-solvent ratio 1:40) for 1 min using a vortex mixer (GO:MIXER MX-S, Camlab, England). Then, the tubes were immersed in an ultrasonic bath (Fisher Scientific, FS30D, Mexico) at 40.00 ± 0.52 °C for 50 min. The mixture was centrifuged (Thermo Scientific, Heraeus™ Fresco™ 21 Microcentrifuge, UK) at 9500 rpm for 3 min and filtered through a 0.45-µm polyvinylidene fluoride (PVDF) filter (GE Healthcare, Whatman™, Uniflo™, USA). All extractions were performed in triplicate.

Table 1 Description of six different compositions of DES tested

DES	Acronym	HBA:HBD molar ratio	References
Choline chloride:glycerol:water	CGH2	(1:2:2)	[35][a]
Choline chloride:glycerol:water	CGH	(1:2:1)	[36]
Choline chloride:glycerol	CG	(1:3)	[37]
Choline chloride:xylitol:water	CXH	(2:1:3)	[38]
Choline chloride:1,2-propanodiol:water	CPH	(1:1:1)	[38]
Choline chloride:oxalic acid:water	COAH	(1:1:1)	[33][a]

[a]With some modifications

2.5 Solvent selection: COSMO-SAC approach

In this work, COSMO-SAC was applied using JCOSMO software developed by Gerber and Soares [22], with the GMHB1808 multi-hydrogen bond parametrization, available for free at (https://doi.org/10.5281/zenodo.3613786) [23]. The sigma profiles were obtained using the GAMESS Quantum Chemistry package [24] following the procedure described by Ferrarini et al. [25]. In this case, cholinium chloride was treated by its ions. After, the prediction of quercetin activity coefficient in different solvents at 298.2 K was also evaluated.

2.6 Experimental design

A three-factor (time, temperature, and solid–liquid ratio) and three levels (− 1, 0, and + 1) face-centered cubic design (FCD) were used to determine the optimal experimental conditions for the total phenolic content and antioxidant activity based on DES selection. The FCD consisted of 17 experimental runs (6 at axial and 8 at the factorial point), including three replications at the center point. The three independent variables were extraction time (X1, min), extraction temperature (X2, °C) and solid-to-solvent ratio (X3, w:v), while the dependent variables (response variables) were the total phenolic content (TPC, mg_{QE}/g), and antioxidant activities by FRAP ($mMol_{TEAC}$/100 g), DPPH free radical scavenging ($mMol_{TEAC}$/100 g), and ABTS ($mMol_{TEAC}$/100 g) methodologies. The variables were coded, as shown in Table 2.

The FCD generation, as the evaluation was performed using Statistica® software (version 10.0, StatSoft). Experimental data were fitted to a second-order polynomial model (Eq. 1) containing the coefficient of linear, quadratic, and interaction terms. Analysis of variance (ANOVA) with a 95% confidence level was carried out for each response variable to evaluate the model's significance and suitability. The experimental data were fitted as a generalized second-order polynomial model according to Eq. 1.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \beta_i x_i + \sum \sum_{i<j=1}^{3} \beta_{ij} x_i x_j \quad (1)$$

where β_0, β_i, β_{ii}, and β_{ij} are the second-order model coefficients for the intercept, linear, quadratic, and interaction terms, respectively, and x_i and x_j are the independent variables. The significance of all the terms of the polynomial equation was analyzed statistically by computing the F value at $p < 0.05$.

Table 2 Independent variables and their levels

Independent Variable	Code units	Level		
		− 1	0	+ 1
Time (min)	X1	10	50	90
Temperature (°C)	X2	25	40	55
Solid-to-solvent ratio (g:mL)	X3	1:100	1:20	1:11

2.7 Extract characterization

The total phenolic content (TPC) was determined by the Folin–Ciocalteu method [26] with minor modification. TPC was expressed as milligrams of quercetin equivalents per grams dry weight of the sample (mgQE/g). The antioxidant activity was determined by three different in vitro assays: ABTS, DPPH, and FRAP methods. The results were expressed as mean ± standard deviation in mmol of Trolox equivalent antioxidant capacity per 100-g dry weight of the powdered macela ($mMol_{TEAC}$/100 g). The $ABTS^{\bullet+}$ assay was performed as described by Arnao and co-workers [27]. The free radical scavenging activity was determined using the stable $DPPH^{+}$ radical based on the Brand–Williams and co-workers [28] method with some adjustments. The ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain [29] with some adaptations. The complete methodologies description is shown in Supplementary Material.

2.8 Chromatographic analysis of phenolic compounds in optimum conditions

The separation and quantification of phenolic compounds from the macela extracts obtained in the optimized conditions were performed by high-performance liquid chromatography (HPLC). The method applied was based on that of Belmiro et al. (2017) with adaptations. The detailed methodology and method validation are shown in Supplementary Material.

2.9 Statistical analysis

The experimental data were presented as the mean ± standard deviation (SD) of the triplicates. Analysis of variances (ANOVA) followed by multiple comparisons test (Tukey's test) and a p-value of ≤ 0.05 was considered significant. Data were presented as mean ± standard deviation (SD), and the experimental design was evaluated using the Shapiro–Wilk normality test. The homogeneity of variances was tested by the Levene test. The statistical analyses were performed using the Statistica® software (version 10.0, StatSoft).

3 Results and discussions

3.1 Solvent selection: in silico and experimental approach

As described in the Sect. 2, the evaluated DES (Table 1) were selected considering those found in the literature that

showed a higher affinity with the quercetin, the main phenolic present in macela. To confirm the quercetin-solvent affinity, the sigma profile (σ-profile) of the quercetin and all DES formers (HBA and HBD) were obtained by the COSMO-SAC model as shown in Fig. 1.

The σ-profile is a tool that allows to determine the charge distribution on a molecule and correlate it with its polarities. Usually, the entire σ-profile of some biomolecule around ± 0.02 e/Å^2. In this profile, the polarity regions can be divided in the following way: regions lower than -0.01 and higher than $+0.01$ e/Å^2 are related to the polar region. In the same way, the region between ± 0.01 e/Å^2 characterized the apolar region of the determined biomolecule. Concerning the color distribution on the biomolecule structure, related to the COSMO cavities, it is important to highlight that deep blue color corresponds to the strongly negative polarization charge density, i.e., positive parts of the biomolecules, while green and red are related to the neutral parts and strongly positive polarization charge density (negative parts of the molecule), respectively. Evaluating the quercetin's σ-profile, it is possible to note that there is a large non-polar region and a lower percentage of polar region. Concerning the constituents used as HBA and HBD the non-polarity, the following order was obtained: glycerol ≈ 1,2-propanodiol > xylitol > oxalic acid > ChCl. It is known that the solute–solvent similarity is a key factor in extraction/solubilization processes, so through the σ-profile, evaluation is expected that higher indexes of quercetin being obtained as more hydrophobic are the evaluated HBD.

In another in silico approach to estimate the solute–solvent affinity by quantum chemistry-based thermodynamic models (COSMO-SAC), the predicted activity coefficients of components in a liquid mixture were obtained. In this work, the activity coefficients of quercetin in all evaluated DES were calculated at 298 K, as shown in Fig. 2. As more negative is the $ln\ \Upsilon$ values, the higher is the affinity solute–solvent. Considering the activity coefficient in infinity dilution ($ln\ \Upsilon^{\infty}$) the following order was obtained: CPH > CG > CGH > CGH2 > CXH > COAH.

Finally, considering that the COSMO model is not able to predict some solvent characteristic, such as viscosity, for example, all proposed DES were also experimental evaluated to obtain phenolic compounds from *A. satureioides*, as shown in Table 3.

As expected, considering the σ-profile of the evaluated HBD, those DES composed of glycerol and 1,2-propanediol presented the higher index of TPC and DPPH. However, to compare the predicted behavior with the experimentally obtained some aspects needs to be highlighted. Firstly, the TPC and DPPH index involves all extracted phenolics, even those with a hydrophilic character, and not only the quercetin. Besides, the model is not able to consider the more difficult mass transfer in high viscosity systems. So as important as the solvent–solute affinity, the DES physical–chemical properties also have some effect in extraction processes.

Based on TPC and DPPH results, the best extraction efficiency was obtained with COAH and CGH2, followed by CPH > CGH > CG > CXH. For all evaluated DES, the

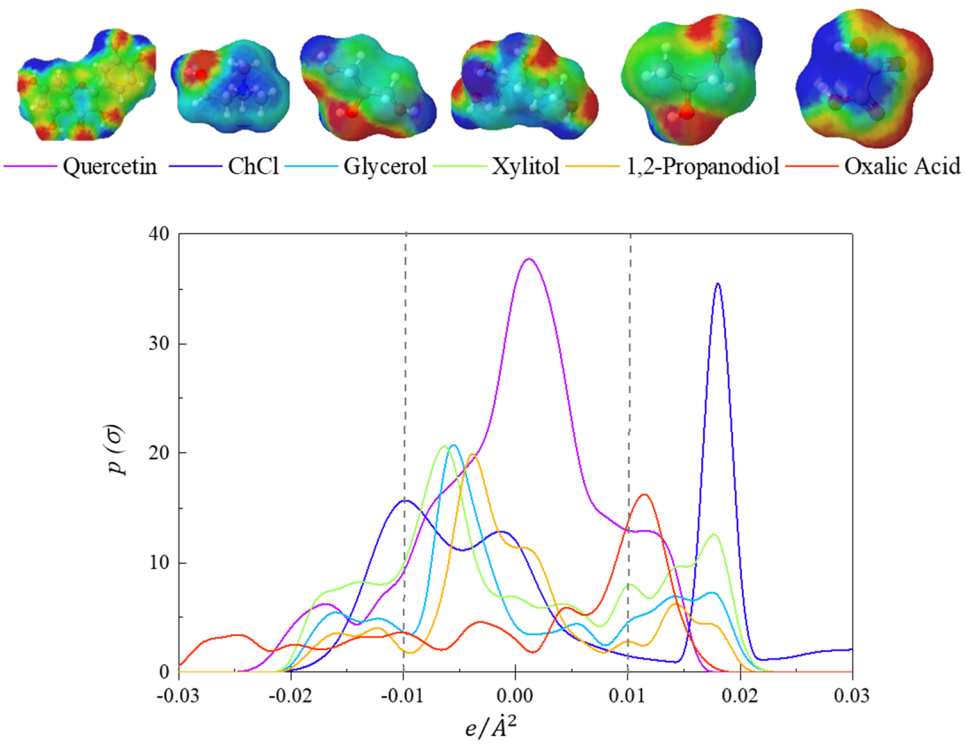

Fig. 1 The σ-profiles and 3D induced surface charge densities obtained from COSMO-SAC of quercetin and each HBA and HBD investigated in this work

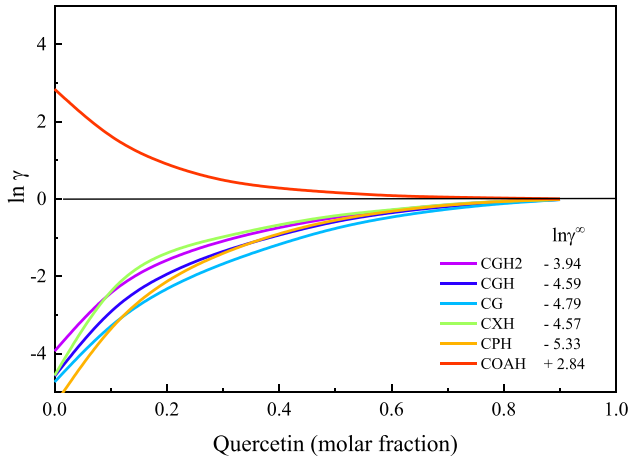

Fig. 2 Activity coefficients (*ln* Υ) of quercetin in all evaluated DES at 298 K and activity coefficient in infinity dilution (*ln* Υ^{∞})

Table 3 Comparison of the antioxidant activity and TPC of six different DES. The antioxidant activity (DPPH) and the total phenolic content (TPC) were expressed as a mean ± SD

Solvent	DPPH (mM_{TEAC}/100 g)	TPC (mg $_{QE}$/g)	DPPH/TPC[a]
CGH2	**20.22[a] ± 2.00**	**2.52[a] ± 0.28**	8.01
CGH	11.71[c] ± 2.34	1.66[b] ± 0.10	7.04
CG	12.26[c] ± 1.94	1.51[b] ± 0.33	8.10
CXH	12.18[c] ± 1.41	0.70[c] ± 0.15	17.29
CPH	17.20[b] ± 0.75	1.73[b] ± 0.35	9.93
COAH	22.25[a] ± 0.14	2.64[a] ± 0.38	8.43

Values obtained through one-way ANOVA

Different letters in the same column represent differences between the samples according to the Tukey test ($p \leq 0.05$)

[a]Ratio between antioxidant activity and total phenolic content

addition of water into the system improved the extraction efficiencies due to the decreases in their viscosity and surface tension, favoring the mass transfer process. Being the quercetin, the target compound on this work, COAH seems not to be a satisfactory solvent, considering the low hydrophobic character of both, HBA and HBD, and low predict affinity with the quercetin. The high values of TPC and DPPH for this solvent probably occur due to extensive extraction of other phenolics compounds, which are more polar than quercetin. Considering that solvent composed of 1,2-propanediol and glycerol as HBD, the results agree with those predicted by the COSMO model, achieving satisfactory extraction (high TPC and DPPH values). Among these solvents, the CGH2 needs to be highlighted considering that presents a good affinity with the quercetin, according to the *ln* Υ prediction, and results in a rich extract in terms of TPC and DPPH. The advantage of this solvent in front of the others evaluated is probably the double benefit conferred by the water addition, i.e., the improvement of the mass transfer because of the viscosity reduction and the simultaneous extraction of quercetin and another more polar phenolic compounds, which can also be of interest.

So considered the expose, the CGH2 was selected for the optimization step of quercetin extraction from *A. satureioides*. It was also taken into consideration that glycerol is a versatile renewable raw material mainly used in chemical and food industry applications such as humectant, solvent, sweetener, and preservative. Besides that, there is a necessity of the search for new applications due to the increased availability of biodiesel that has been boosted the world market for glycerol [30]. These features are essential to selecting a solvent to be used as economically competitive and environmentally friendly. It should also be highlighted with the fact that the extracts obtained from CGH2 extraction could be directly used in products for human consumption without the need for an expensive downstream purification step, affording good prospects in the manufacturing of functional polyphenol-rich extracts. Despite the described limitations of COSMO model, the solvent screening predicted by COSMO-SAC allows a better comprehension concerning the solute–solvent affinity in these systems.

3.2 Effect of process variables on antioxidant activity (ABTS, DPPH, and FRAP) and TPC by FCD

As previously mentioned, CGH2 was selected as the most promising solvent for the extraction of phenolic compounds from *A. satureioides*. Seventeen runs were carried out according to the experimental designs described in Sect. 2.5. The responses of total phenolic content (TPC) and antioxidant activities (ABTS, DPPH, and FRAP) for each run of the experimental design are presented in Table 4.

As shown in Table 4, antioxidant activity values varied from 11.13 ± 1.92 (run 5) to 65.14 ± 3.45 (run 4) $mMol_{TEAC}$/100 g of dry sample for ABTS assay, 6.19 ± 0.36 (run 5) to 39.50 ± 1.31 (run 4) $mMol_{TEAC}$/100 g of dry sample for DPPH assay, and 2.66 ± 0.08 (run 4) to 28.77 ± 2.75 (run 4) $mMol_{TEAC}$/100 g of dry sample for FRAP assay.

The experimental results were mathematically evaluated, and after building a quadratic model by applying second-order polynomial equations without data transformation (Eqs. 2–5), the predicted values were obtained (Table 4).

$$Y_{ABTS} = 28.45 + 9.11X_3^2 + 7.59X_2 - 15.99X_3 \quad (2)$$

$$Y_{DPPH} = 16.44 - 2.97X_1^2 + 7.52X_3^2 + 2.39X_1 + 7.75X_2 - 5.80X_3 - 1.26X_2X_3 \quad (3)$$

$$Y_{FRAP} == 11.91 + 2.57X_3^2 + 3.22X_1 + 7.20X_2 + 1.32X_1X_2 \quad (4)$$

Table 4 Face-centered cubic design of three variables (time, temperature, and solid-to-solvent ratio) with the observed responses (mean ± SD) from the experimental design for face-centered design and their respective predicted values obtained from model equations

Run	Coded values			Independent variables			Responses				Predicted			
	X1	X2	X3	Time	Temperature	Solid-to-solvent ratio	TPC	ABTS	DPPH	FRAP	TPC	ABTS	DPPH	FRAP
	(min)	(°C)	(w:v)	(min)	(°C)	(w:v)	(mg_{QE}/g)	(mM_{TE}/100 g)	(mM_{TE}/100 g)	(mM_{TE}/100 g)	(mg_{QE}/g)	(mM_{TE}/100 g)	(mM_{TE}/100 g)	(mM_{TE}/100 g)
1	−1	−1	−1	10	25	1:100	$2.89^{b,c,d} \pm 0.24$	$37.27^{d} \pm 4.29$	$16.22^{b,c} \pm 0.08$	$2.66^{i} \pm 0.08$	2.99	41.56	15.40	5.38
2	+1	−1	−1	90	25	1:100	$2.52^{c,d,e} \pm 0.18$	$48.84^{c} \pm 8.06$	$20.11^{b,c} \pm 3.06$	$8.61^{f,g,h} \pm 0.14$	3.06	50.07	22.69	9.18
3	−1	+1	−1	10	55	1:100	$3.33^{b,c} \pm 0.63$	$52.92^{b,c} \pm 4.87$	$34.25^{a,b} \pm 9.82$	$17.89^{c} \pm 0.83$	4.15	57.04	30.89	17.14
4	+1	+1	−1	90	55	1:100	$6.33^{a} \pm 0.38$	$65.14^{a} \pm 4.94$	$39.50^{a} \pm 1.31$	$28.77^{a} \pm 2.75$	6.09	65.56	38.18	26.23
5	−1	−1	+1	10	25	1:11	$1.01^{f} \pm 0.08$	$11.13^{h} \pm 1.92$	$6.19^{b,c} \pm 0.36$	$5.94^{h,i} \pm 0.51$	1.48	9.57	6.30	5.38
6	+1	−1	+1	90	25	1:11	$1.58^{e,f} \pm 0.11$	$16.19^{g,h} \pm 1.60$	$9.42^{c} \pm 0.30$	$8.32^{g,h} \pm 0.66$	1.55	18.08	8.57	9.18
7	−1	+1	+1	10	55	1:11	$2.96^{b,c,d} \pm 0.34$	$25.78^{e,f,g} \pm 2.80$	$18.34^{b,c} \pm 1.11$	$17.02^{c} \pm 0.49$	2.64	25.06	21.80	17.14
8	+1	+1	+1	90	55	1:11	$3.89^{b} \pm 0.21$	$28.55^{d,e,f} \pm 2.21$	$24.65^{a,b,c} \pm 0.75$	$25.07^{b} \pm 0.65$	4.58	33.57	24.06	26.23
9	0	0	0	50	40	1:20	$2.51^{c,d,e} \pm 0.23$	$32.63^{d,e} \pm 0.90$	$17.18^{b,c} \pm 6.90$	$12.60^{d,e} \pm 0.93$	2.56	28.45	16.44	11.91
10	0	0	0	50	40	1:20	$2.54^{c,d,e} \pm 0.36$	$25.07^{e,f,g} \pm 4.73$	$16.75^{b,c} \pm 1.80$	$13.28^{d} \pm 0.26$	2.56	28.45	16.44	11.91
11	0	0	0	50	40	1:20	$2.99^{b,c,d} \pm 0.14$	$30.98^{d,e} \pm 2.15$	$17.71^{b,c} \pm 0.21$	$11.86^{d,e,f} \pm 1.68$	2.56	28.45	16.44	11.91
12	−1	0	0	10	40	1:20	$1.92^{d,e,f} \pm 0.15$	$21.69^{e,f,g,h} \pm 1.72$	$10.47^{c} \pm 0.56$	$9.49^{e,f,g} \pm 0.59$	2.06	24.19	11.07	8.68
13	+1	0	0	90	40	1:20	$2.81^{b,c,d,e} \pm 0.75$	$32.64^{d,e} \pm 3.37$	$15.67^{b,c} \pm 2.71$	$14.48^{c,d} \pm 1.20$	3.06	32.71	15.85	15.13
14	0	−1	0	50	25	1:20	$1.59^{e,f} \pm 0.06$	$18.84^{f,g,h} \pm 2.31$	$9.33^{c} \pm 0.88$	$6.41^{g,g} \pm 0.68$	1.51	20.71	8.69	4.70
15	0	+1	0	50	55	1:20	$3.54^{b,c} \pm 0.55$	$37.30^{d} \pm 3.44$	$22.00^{a,b,c} \pm 1.57$	$15.23^{c,d} \pm 0.87$	3.60	36.19	24.18	19.11
16	0	0	−1	50	40	1:100	$5.29^{a} \pm 1.04$	$63.61^{a} \pm 3.45$	$26.85^{a,b,c} \pm 0.88$	$12.80^{d,e} \pm 2.16$	4.07	53.56	29.77	14.48
17	0	0	+1	50	40	1:11	$3.37^{b,c} \pm 0.10$	$26.19^{d,e,f,g} \pm 2.17$	$20.30^{a,b,c} \pm 0.98$	$17.72^{c} \pm 1.56$	2.56	21.57	18.16	14.48
p (Normality)*							0.00012	0.00050	0.00046	0.0110				
p (Homogeneity)**							0.00001	0.00001	0.00290	0.177817				

Values obtained through one-way ANOVA

Different letters in the same column represent differences between the samples according to the Tukey test ($p \leq 0.05$)

*Values obtained according to the Shapiro–Wilk test (p-value > 0.05 present normal distribution)

**Values obtained according to the Levene test of variances (p-value > 0.05 present same variance)

$$Y_{TPC} = 2.56 + 0.76X_3^2 + 0.50X_1 + 1.05X_2 - 0.75X_3 + 0.47X_1X_2 \quad (5)$$

The coefficient of determination adjusted (R^2 adj) of the model for all response variables was greater than 0.77, indicating that the model fits the experimental data well and is adequate for predictive use within the range of experimental variables in this study. The ANOVA results revealed the interaction patterns between the independent and response variables. TPC is a critical analysis to figure out the amount of phenolic content in the samples since the phenolic compounds are responsible for donating hydrogen and forming stable radical intermediate. In the case of recovery, the phenolic compounds using CGH2 as the solvent, the multiple regression analysis showed that temperature and time significantly affect the TPC. They were considered the main factors involved in the process of extraction. The quadratic coefficient solid-to-solvent ratio (X_3^2) and the linear temperature coefficient (X_2) were significant ($p < 0.05$) for all response variables analyzed (ABTS, DPPH, FRAP, and TPC; Eqs. 2–5) and affected positively the antioxidant activity of the extracts. For DPPH, FRAP, and TPC models, the extraction temperature was the main factor that influences to increase the extraction of bioactive compounds from macela. Also, the linear solid-to-solvent ratio coefficient X_3 presented a negative effect, except in the FRAP model. Although high temperatures are a critical parameter in extraction to avoid degradation, for all the evaluated antioxidant assays, the temperature was the factor that had the most significant and positive effect on antioxidant activity.

According to results, we could observe that DES can stabilize and keep the bioactive compounds even under more extreme extraction conditions, such as a temperature of 55 °C. Two main aspects may be correlated with this affirmation: DES composition and viscosity. The first can be supported by the observations in nature that the living cells may survive by forming DES under drought conditions, at high or low temperatures, in order to preserve the membranes, enzymes, and metabolites, such as biocompounds [31]. The second is due to an increase in the diffusion coefficient caused by the increase in temperature, which decreases the viscosity of the solvent and increases the solubility of biomolecules in DES [32]. In this way, reduction of surface tension occurs, as well as a decrease in the interaction between biocompounds and the macela matrix, favoring the extraction process and leading to enhanced desorption and dissolution of the target component in the DES [33]. Therefore, although requiring a higher temperature for the extraction of the phenolic compounds from macela, this variable did not affect the stability of these compounds, highlighting the protective effect of DES.

Finally, the extraction time, which means the time of contact between the solvent and the matrix, showed significant effects on the phenolic content. It suggests that there is a gradual release of solute from the solid matrix to the solvent during the extraction time interval. Thus, it was observed that the antioxidant activity was maintained even in relatively high times of extraction. It is known that not only the temperature but also the extraction time can lead to degradation of the antioxidants. This behavior is also correlated to the hydrogen bonds formed between the extract and the target compounds. Also, the viscosity of DES reduces oxidative degradation by decreasing the movement of solute molecules.

As shown in Fig. 3, as well as in Figure S1 (*cf.* Supplementary Material), these results are also reinforced by the desirability function, which increases when temperature and time increase while the solid-to-solvent ratio decreases. The optimal experimental conditions, equivalent to maximum desirability ($D = 0.9093$) using CGH2 (1:2:2, choline chloride:glycerol:water) to extract the biocompounds from macela, was achieved at 55 °C, 90 min, and 1:100 solid-to-solvent ratio. At those optimal recommended conditions, it was predicted that the maximum average to antioxidant activity using ABTS, DPPH, and FRAP would be 65.56 ± 7.21, 38.18 ± 0.76, 26.23 ± 1.84 mM_{TEAC}/100 g, respectively, and the total phenolic content TPC 6.09 ± 0.91 mg/g.

3.3 Phenolic profile by HPLC analysis

The contents and identification of phenolic acids and flavonoids in the optimized extract (90 min at 55 °C and solid-to-liquid ratio 1:100 (w/v)) from *A. satureioides* were determined by HPLC analysis. The validation parameters of HPLC methodology are described in Table S1. Standard calibration curves presented good linearity based on regression analysis ($R^2 > 0.9972$). The LOD and LOQ values of the individual analytes clearly showed that the analytical method had excellent sensitivity.

According to the applied methodology, four of the eight standard compounds were identified, as described in Table 5. Among the four, two of them were the ones that stood out the most, being quercetin and catechin, 27.7 mg/100 g and 26.1 mg/100 g, respectively. Both are flavonoids and are linked to the anticarcinogenic and anti-inflammatory macela properties. The higher quercetin concentration on the extract reinforces the applicability of the in silico tools to predict the solute–solvent affinity. Phenolic acids, p-coumaric, and Rosmaniric were found in lesser amounts, and these also have anti-inflammatory properties. Both phenolic acids have not been previously reported in *A. satureioides*.

When compared to organic solvents [34], the extract obtained with DES presented a capacity about two times greater to extract quercetin, the main flavonoid of the macela, while the catechin extraction was around 1600 times

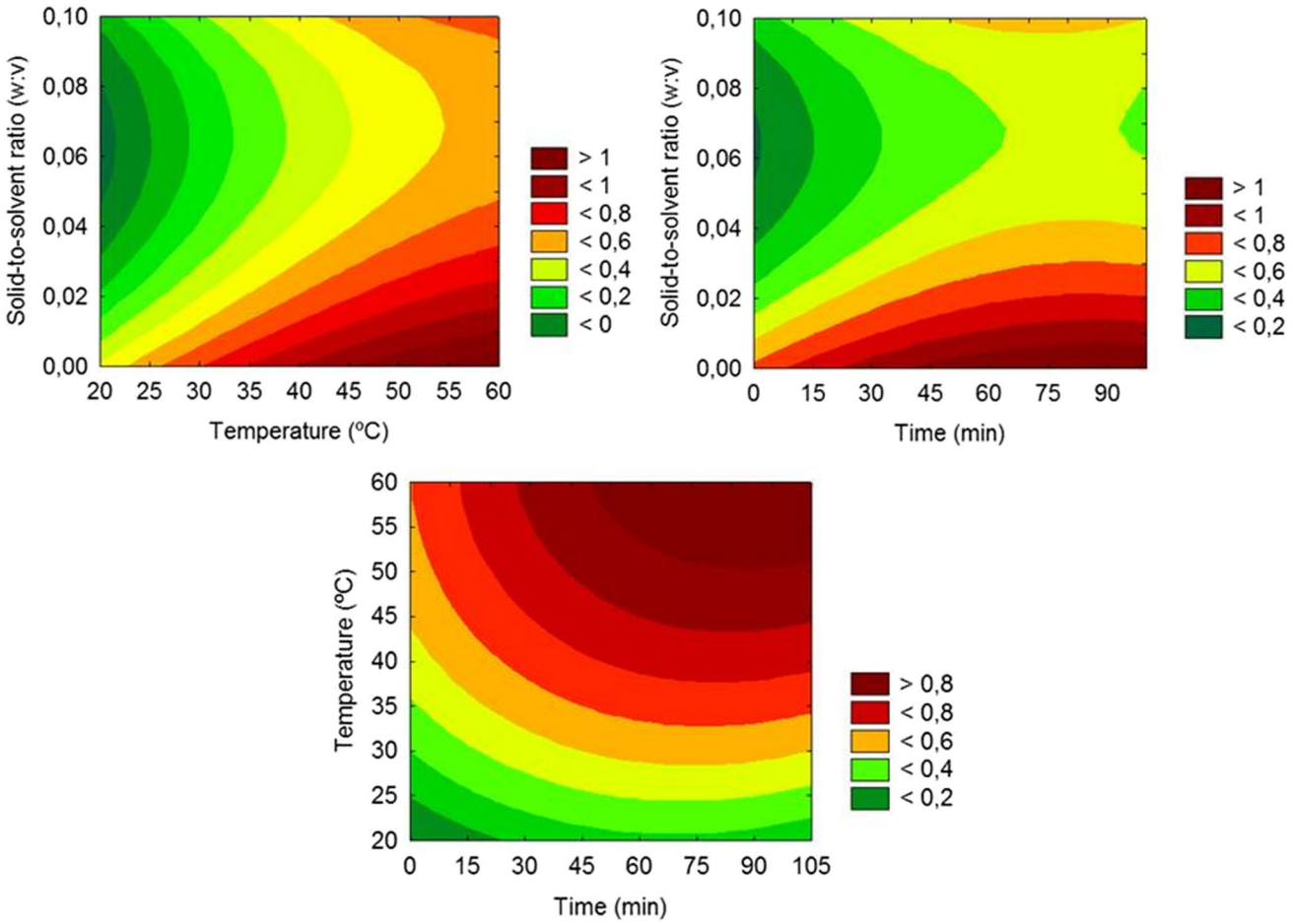

Fig. 3 Contour plots illustrating the effect of simultaneous variation of the three independent variables considered: time (10–90 min), temperature (25–55 °C), and the solid-to-solvent ratio (1:100–1:11)

Table 5 Phenolic compounds found in macela extract obtained by using CGH2 in the optimal conditions [90 min at 55 °C and solid-to-liquid ratio 1:100 (w/v)]

Chemical standards	Concentration (mg/100 g)
Catechin	26.10 ± 0.90
Caffein	n.d
Rutin	n.d
p-Coumaric acid	18.80 ± 0.90
Ferulic acid	n.d
Naringin	n.d
Rosmaniric acid	12.56 ± 0.1
Quercetin	27.00 ± 0.26
Catechin[a]	$1.57.10^{-2} \pm 0.01$
Quercetin[a]	14.86 ± 0.12

[a]Using 50% (v/v) acetone at 25 °C and a 1:40 solid-to-liquid ratio (w/v) by means at an ultrasound at 50 min

greater. This higher extractive capacity is related to the hydrogen bonds formed between CGH2 and the target component, as well as with the polarity of this solvent, which favors the dissolution of these components in the extractive medium.

4 Conclusion

The present work demonstrated that DES aided by UAE could be applied for the green recovery of valuable compounds from *A. satureioides*. Among DES evaluated by COSMO model and experimental approach, CGH2 (1:2:2) was selected as the most efficient. The optimization of the extraction procedure was achieved employing a face-centered cubic design in which the optimal conditions were 55 °C, 90 min, and the solid-to-solvent ratio of 1:100 (w/v). These conditions provided an extract with a higher concentration of natural phenolics compounds, besides showing the DES ability to preserve the antioxidant activity even under

high extraction temperature conditions. According to HPLC analysis, the extracts using DES presented concentrations of flavonoids that are much higher than that found in the extracts obtained with organic solvents. Therefore, DES presents great potential as solvents to extraction of bioactive compounds from vegetable sources, once it can efficiently extract similar compounds as alcoholic solvents, taking in advantage to be better for direct consumption and enhanced stabilization of the bioactive compounds.

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Declarations

Conflict of interest The authors declare no competing interests.

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