ACS Sustainable Chemistry & Engineering

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ACS Sustainable Chem. Eng., **Just Accepted Manuscript** • DOI: 10.1021/acssuschemeng.0c03553 • Publication Date (Web): 21 Jul 2020

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

USING COSMO-RS IN THE DESIGN OF DEEP EUTECTIC SOLVENTS FOR THE EXTRACTION OF ANTIOXIDANTS FROM ROSEMARY

José Pedro Wojeicchowski[†,‡], Ana M. Ferreira[‡], Dinis O. Abranches[‡], Marcos R. Mafra,[†] and João A.P. Coutinho[‡*]*

[†] Department of Chemical Engineering, Federal University of Paraná (UFPR), Polytechnic Center, 81531-990, Brazil.

[‡] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro (UA), 3810-193, Portugal.

*Corresponding authors: marcos.mafra@ufpr.br (Phone: +55 3361-3586) and jcoutinho@ua.pt (Phone: +351 234 370 200)

ABSTRACT

Trial and error remain the most common method to select the right solvent for the extraction of natural products, in particular when dealing with novel, poorly studied solvents such as deep eutectic solvents (DES). This can lead to either a lengthy or a suboptimal selection of solvents. COSMO-RS, a quantum chemistry-based thermodynamic model, was applied in this work to screen the best DES for the extraction of carnosic acid and carnosol, present in rosemary. Twenty-eight hydrogen bond acceptors (HBA) and forty-nine hydrogen bond donors (HBD) were selected for the initial computational evaluation that revealed that hydrophobic DES formed by ammonium chlorides as HBA, and the fatty acids, aromatic carboxylic acids or alcohols as HBD to be the most suitable solvents for this extraction. Then, solid-liquid extractions were performed using these solvents to identify the best one and to optimize its composition. The ability of the solvents to obtain an extract rich in carnosic acid and carnosol was measured by the antioxidant activity of the extract, being the mixture of 15 wt% of tetrapropylammonium chloride, 55 wt% of 1,2-propanediol and 30 wt% of water the solvent with the best performance. A response surface methodology was then carried out to optimize extraction conditions, in which antioxidant activity up to 85 mg TE/g dw, that correspond to the extraction of 14.8 mg of carnosic acid and 18.9 mg of carnosol /g dw. Finally, the antioxidant activity of extracts obtained from a solvent without HBA was investigated. These aqueous mixtures of 1,2-propanediol showed a small loss in the performance. However, since the extraction process is simplified and this alcohol is biocompatible and allowed in formulations, the binary mixture seems to be a good, more sustainable alternative, to the ternary one.

KEYWORDS: *Rosmarinus officinalis L.*, hydrophobic DES, carnosic acid, carnosol, antioxidant activity, solid-liquid extraction, activity coefficient.

INTRODUCTION

Antioxidants have an important function in extending food shelf-life because of their role in lipid oxidation control, that is the most important cause of food spoilage after the damage by microorganisms.[1] The most commonly used antioxidants in the food industry are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Even though these synthetic antioxidants are efficient at slowing down the oxidative deterioration of lipids, they have been shown to act as tumor initiators or promotors in animal tissue, [2,3] suggesting some degree of carcinogenicity. One way to mitigate the health risk associated to the continued consumption of BHA or BHT is to identify more harmless alternatives, such as using natural extracts, considered safe.[4] Furthermore, consumers are looking for “free from” artificial additives/ingredients food products, which increased the *clean label* products demand and research on this issue.[5]

Rosemary (*Rosmarinus officinalis* L.) is an aromatic herb originating from the Mediterranean region and cultivated worldwide. Extracts of rosemary possess high antioxidant activity, brought about by the presence of phenolics diterpenes, namely carnosic acid and carnosol, which account for 90% of the antioxidant activity of rosemary.[1,6] Due to their low toxicity and natural origin, rosemary extracts were allowed to be used as food additives (E 392) by the European Food Safety Authority (EFSA) in 2008, with an adult daily limit of 0.3 mg of rosemary extract per kg of body weight.[7] There are commercial rosemary extracts in different forms available for food application as antioxidant in i) oils and fats, ii) bakery products, iii) dressing and mayonnaise, iv) meat and poultry products, v) potato flakes and as preservatives, inhibiting or slowing down microorganisms activity.[4,8,9]

Rosemary extracts are currently prepared from dried rosemary leaves by using volatile organic solvents, such as hexane, chloroform, acetone, methanol or ethanol.[4] To replace these volatile and hazardous organic solvents, deep eutectic solvents (DES) and their aqueous solutions have emerged as an alternative.[10] Nowadays, DES have been used for the extraction of bioactive compounds, like antioxidants, from natural resources; *e.g.,* phenolics compounds from mulberry[11] and Chlorella vulgaris. [12]

First introduced by Abbott and co-workers,[13,14] DES are mixtures of solid components for which the eutectic temperature is substantially lower than that predicted by considering an ideal liquid mixture.[15] DES are systems formed by a eutectic mixture of Lewis or Brønsted acids and bases,[16] usually prepared by combining at least one hydrogen bond donor (HBD), such as alcohols or carboxylic acids and one hydrogen bond acceptors (HBA), such as quaternary ammonium salts.[14,17] Due to their nature, DES are designer solvents, since a careful choice of their constituents may lead to a set of specific properties required for a given application.[18] This versatility has contributed to the successful application of DES in solid-liquid extraction of target bioactive compounds. [19–25] Despite all DES reported in the literature involving solid-liquid extraction, there are yet many others that have not been evaluated, due to the enormous number of possible combinations between HBA and HBD. Therefore, the use of models as a pre-screening tool to overcome the time and money-consuming experimental measurements by a trial and error approach must be encouraged.

The COnductor-like Screening MOdel for Real Solvents, (COSMO-RS), is a thermodynamic model based on quantum chemistry and statistical thermodynamics that can predict the chemical potential of individual compounds in liquid mixtures.[26–28] COSMO-RS is useful for the *a priori* design of DES by i) predicting the solid-liquid phase diagram of the solvent, including its eutectic temperature,[26,29] and ii) predicting the solubility of biomolecules in DES, such as terpenoids from citrus essential oil, [30] limonene from orange peel waste, [31] and hydroxytyrosol from olive leaves.[32]

In this work, DES are used to extract carnosol and carnosic acid from rosemary. Owing to the numerous possible combinations of substances to form DES, COSMO-RS is used as an initial screening tool to select the most promising solvents, avoiding the experimental trial and error methodology. The impact of the water content of DES on the solubility was also studied. The best solvents were selected from the initial screening and were used to extract carnosol and carnosic acid from rosemary experimentally. Acetone and ethanol were also used for comparison purposes. Then, the composition of the two best solvents was optimized using a mixture design, *i.e.*, the percentage of each compound (HBA, HBD and water) was optimized. Finally, the response surface methodology (RSM) was applied to optimize the extraction operational conditions of the best DES, namely temperature, liquid-solid ratio (L-S ratio) and time of extraction. Computational and experimental methods were thus here combined, to reduce the number of experiments necessary to develop a process to obtain extracts rich in carnosic acid and carnosol, with a high antioxidant activity of rosemary leaves using DES and solid-liquid extraction.

MATERIALS AND METHODS

Materials

Rosemary leaves were collected from UFPR's Canguiri Experimental Farm (25°23'12.3"S 49°07'33.3"W). The material was hand-selected and dried in an oven at 40 C, with air circulation, until weight variation was no longer detectable. The dried leaves were grounded in a laboratory mill (Requipal, MR 320, São Paulo, Brazil), sieved to obtain rosemary powder with particle size smaller than 0.2 mm, vacuum packed and frozen for further application at -10 °C.

The HBA tetraethylammonium chloride ($N_{2222}Cl$), tetrapropylammonium chloride ($N_{3333}Cl$) and tetrabutylammonium chloride ($N_{4444}Cl$) were supplied by Sigma-Aldrich. The HBD 1,2-propanediol and 5-phenylvaleric acid were purchased from Sigma-Aldrich, propionic acid from Acros Organics and ethylene glycol from Panreac. All the reagents have a purity higher than 97.0%.

Methods

DES's components list and preparation

A database of DES commonly used in the extraction of bioactive compounds from natural sources was created based on literature reports.[17,22,33–40] The database is composed of twenty-eight hydrogen-bond acceptors and forty-nine hydrogen-bond donors, as shown in the Supporting Information, Tables S1 and S2.

The various DES tested experimentally were prepared by heating the HBA-HBD mixture at 70 °C with continual stirring until a homogeneous transparent liquid was obtained.

COSMO-RS simulations

To use COSMO-RS, the geometry and charge density of the individual molecules of a system need to be optimized using DFT. In this work, each molecule was optimized using the COSMO-BP-TZVP template of the TmoleX software package[41] (interface of TURBOMOLE), which includes a def-TZVP basis set, DFT with the B-P83 functional level of theory and the COSMO solvation model (infinite permittivity). All COSMO-RS calculations were performed using the software COSMOtherm[42] with the BP_TZVP_C30_19.ctd parametrization. As COSMO-RS is not suitable for calculations with ionic species,[28] quaternary ammonium salts applied as HBA were dealt as ion pairs and then optimized using TmoleX.[26] DES were treated as binary mixtures of HBD and HBA at a fixed stoichiometric rate within the framework of COSMO-RS.[43] With this approach, a vast number of DES is accessible without additional quantum chemical calculation, that is especially relevant for DES screening. [44]

For any compound, its solubility in a solvent is inversely proportional to its activity coefficient in the system. As such, COSMO-RS was used to predict the activity coefficient of carnosic acid and carnosol in 1372 DES at 35 ºC and infinite

157 dilution. The components of the DES were present in an equal molar ratio, and a
158 water contents of 30 wt% was also tested.

159

160 *Solid-liquid extractions*

161 Solid-liquid extractions of antioxidants from rosemary powder were carried out
162 using the most promising DES obtained by COSMO-RS. Moreover, the solvents
163 used in the extraction were pure DES or aqueous solutions of DES with different
164 water content (10 and 30 wt%). The extraction solvent was prepared
165 gravimetrically within 10^{-4} g. In these experiments, the stirring was kept constant
166 at 600 rpm, the temperature at 35 ± 0.5 °C and the liquid-solid ratio (L-S ratio,
167 weight of solvent per weight of dried rosemary powder) at 20:1. For comparison
168 purposes, water, pure acetone and water-ethanol mixture (with 30 wt% of water)
169 were used as controls for the extractions. After the extraction step, the solvents
170 were separated from the biomass by centrifugation (at 4000 rpm for 30 minutes
171 using an Eppendorf centrifuge 5804), and the supernatant was filtered using a
172 0.20 μm syringe filter.

173

Antioxidant activity assay

The antioxidant activities of the extracts were evaluated using the 2,2-diphenyl-1-picrylhyldrazyl radical ($DPPH^•$). The widely used and rapid method of Brand-William consists of the stabilization of the free radical $DPPH^•$ by the action of an antioxidant.[45,46] The color change of the reagent solution from purple to yellow was monitored by visible spectroscopy at 517 nm. The antioxidant activity was calculated using a standard curve (mg Trolox/L = 441.8 × %AA – 16.725; R^2 = 0.99) where %AA is the percentage of $DPPH^•$ reduction, given by equation 1:

$$\%AA = \frac{(ABS_b - ABS_s)}{ABS_b}.100 \quad (1)$$

where, ABS_b and ABS_s are the blank and sample absorbance values, respectively, at 517 nm after 30 min of reaction in the darkness. The antioxidant activity results were converted and expressed in mg of trolox equivalent per g dry weight of rosemary leaves (mg TE/g dw).

HPLC-DAD

The quantification of carnosic acid and carnosol was carried out by HPLC-DAD (Shimadzu, model PROMINENCE). Chromatographic analyses were performed with an analytical C18 reversed-phase column (250 × 4.60 mm), Kinetex 5 µm C18 100 Å, from Phenomenex. The separation was conducted in a gradient system of 0.1% of acetic acid-methanol (phase A) and 0.1% of acetic acid-ultra-pure water, according to de Oliveira and co-workers,[47] with slight modification. The separation was conducted using the following gradient mode: 0 min of phase A; 7 min of phase A; 11 min 80% of phase A; 23 min 80 % of phase A; 24 min 90 % of phase A; 28 min 40 % of phase A; 40 min 40 % of phase A. The flow rate used was 0.6 mL/min, with a volume injection of 10 µL, and DAD was set at 280 nm. The column oven was operated at a controlled temperature of 30 °C. Each sample was analyzed at least in duplicate. Calibration curves were prepared using the pure antioxidants with samples dissolved in acetic acid. Carnosol and carnosic acid displayed a retention time of 24.1 and 32.2 min, respectively. The amount of carnosol and carnosic acid extracted were expressed in mg of weight of extracted compound per g dry weight of rosemary leaves (mg/g dw).

Design of mixture

Mixture design, such as simplex-centroid, allows the investigation of the synergistic or antagonistic effects of the mixture components, and it is a valuable tool to design solvent composition.[48] The independent variables named the hydrogen-bond acceptor ($N_{3333}Cl$), the hydrogen-bond donor (propionic acid or 1,2-propanediol) and water from 15 to 70 wt% were tested in ten different assays, according to the design of experiments (Table S3, Supporting Information). Each extraction was performed using a L-S ratio of 20:1, at 35°C, during 120 minutes under constant magnetic stirring (600 rpm). The supernatant was collected after centrifugation and the antioxidant activity measured.

Response Surface Methodology (RSM)

The RSM was applied to optimize the operational conditions to maximize the antioxidant activity of rosemary extracts. In a 2^k RSM there are *k* different factors that can contribute to a response *y*, according to this polynomial equation 2:

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_i \beta_i X_i^2 + \sum_{i<j} \beta_i \beta_j X_i X_j \quad (2)$$

where β_0, β_i, β_{ii} and β_{ij} are the adjusted coefficients for the intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j are the independent variables. In this study, temperature, time and L-S ratio were submitted to a 2^3 factorial planning to optimize the antioxidant activity, using the solvent composition (30 wt% of water, 15 wt% of HBA and 55 wt% of HBD) previously determined by the design of mixture. Twenty experiments were developed, and the conditions applied are provided in the Supporting Information (Tables S4 and S5). The obtained results were statistically analyzed with a confidence level of 95%, subjected to analysis of variance (ANOVA) and regression analysis using Statistica 7.0 software (StatSoft, Tulsa, OK, USA). Contour plots of the antioxidant activity were generated from adjusted models, and through their analysis, the optimal conditions can be determined.

RESULTS AND DISCUSSION

COSMO-RS Solubility Predictions

The first step of this work was the design of DES to extract carnosic acid and carnosol, the main antioxidant compounds present in rosemary leaves, using COSMO-RS. The σ-profiles of these solutes and of water, which are depicted in Figure 1, show that carnosic acid and carnosol are apolar, as noted by the large peak around 0 $e/\dot{A}^2$, that correspond to the green colored surface.[49] Figure 1 also shows that carnosic acid is slightly more apolar than carnosol, which is in agreement with their octanol/water partition coefficients (log Kow of carnosic acid is 5.13 and of carnosol and 4.58),[50] which is much higher than that of ethanol (-0.29). As such, it is expected that i) the best DES to dissolve carnosol and carnosic acid are those prepared from hydrophobic HBA and HBD, and ii) the addition of water to the DES composition will have a negative impact on their solubility.

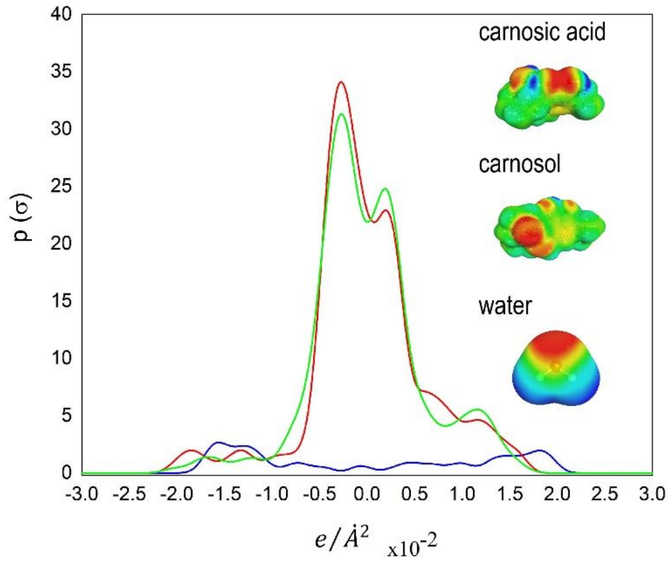

Figure 1. Sigma-profiles of carnosic acid (red), carnosol (green) and water (blue) and their sigma-surfaces representation.

COSMO-RS was applied to predict the activity coefficient of carnosol and carnosic acid at infinite dilution (γ_∞) in all possible combinations of the HBA and HBD studied (1372 possible mixtures considering equimolar combinations of 28 HBA and 49 HBD). These results are depicted in Figure 2 as contour plots, where the color code represents $ln\,\gamma_\infty$ of carnosic acid (Figure 2A) and carnosol (Figure 2B) in the DES, at 35 °C. In each graph, the horizontal axis represents the HBD, and the vertical axis represents the HBA (see Tables S1 and S2 of the Supporting Information for number-compound correspondence).

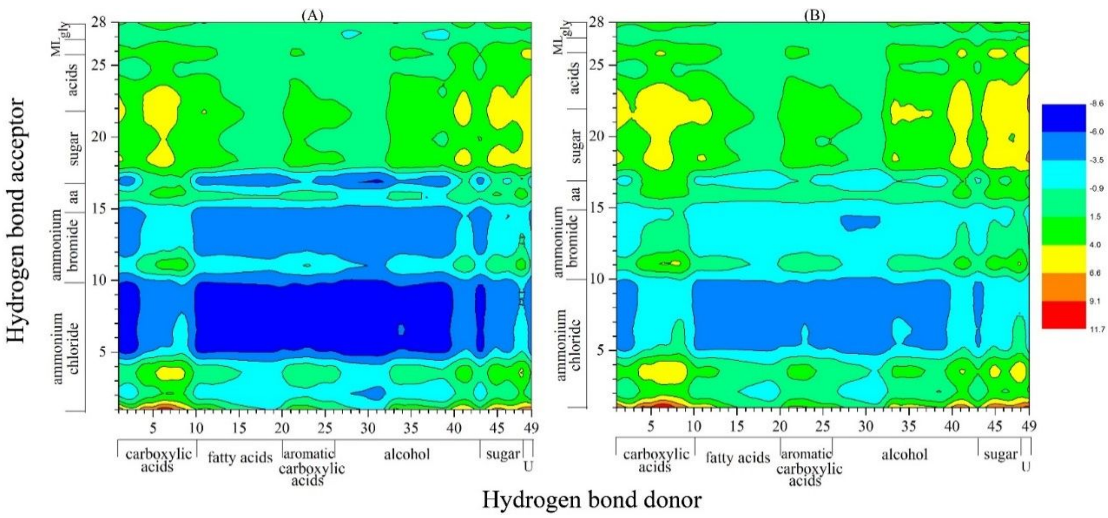

Figure 2. Predicted $\ln \gamma_{solutes}^{\infty}$ in DES (1:1) at 35 °C using COSMO-RS. **(A)** carnosic acid and **(B)** carnosol.

Figure 2 reveals that the solubility of carnosic acid or carnosol is mostly affected by the choice of HBA, since the best results for either solute were obtained for DES composed of ammonium chlorides or ammonium bromides. For example, DES composed of HBA numbered 7 to 10 led to negative $ln\,\gamma_{\infty}$ for carnosic acid and carnosol, regardless of the choice of HBD. In fact, this seems to be connected to the hydrophobicity of the HBA. For instance, $ln\,\gamma_{\infty}$ decreases in the series of ammonium chlorides, from number 1 to number 10, as the hydrophobicity of the HBA increases.

Despite the higher impact of the choice of HBA in the solubility of carnosic acid and carnosol, the selection of a hydrophobic HBD can also have a synergistic effect. For instance, the use of dodecanol (number 30) or cyclohexanol (number 43) leads to a further decrease in $ln\, \gamma_{\infty}$ for both solutes, regardless of the choice of HBA.

It is interesting to note that Figures 2A and 2B are qualitatively identical, in the sense that the best and worst DES to dissolve carnosic acid or carnosol are the same. However, in absolute terms, the predicted $ln\, \gamma_{\infty}$ for carnosol is always slightly higher than that for carnosic acid. Nevertheless, both antioxidants exhibited a high affinity for the solvent in the same contour regions, facilitating the choice of DES to extract both compounds simultaneously.

The achievement of an efficient extraction of antioxidants from rosemary depends not only on the solubility of the solutes in the solvent but also on the solvent properties, namely its viscosity. In general, DES exhibit high viscosity due to an extensive hydrogen-bonding network established between their components,[33] which hampers the use of DES in solid-liquid extraction units. As such, it is common to add a certain amount of water to DES in order to decrease their viscosity, even though, in some cases, it negatively impacts the target compounds solubility.[33,51] As such, the impact of water on the $ln\,\gamma_\infty$ of carnosic acid and carnosol was studied by repeating the aforementioned procedure considering a water content of 30 wt% in the eutectic solvent. These results are reported in Figure 3 using contour plots analogous to Figure 2. Note that Figures 3-4 are available ungrouped in the Supporting Information, Figures S1-S4.

Figure 3 is qualitatively identical to Figure 2, but with a clear, systematical increase in the value of $ln\,\gamma_\infty$ for both solutes, regardless the choice of DES. In other words, the addition of water to the DES studied negatively impacts the solubility of carnosic acid and carnosol, which is to be expected since these solutes are highly hydrophobic, as mentioned above. Nevertheless, this increase in the value of $ln\,\gamma_\infty$ is not very significant and does not change the conclusions reached above regarding the best DES components to solubilize these carnosic acid and carnosol.

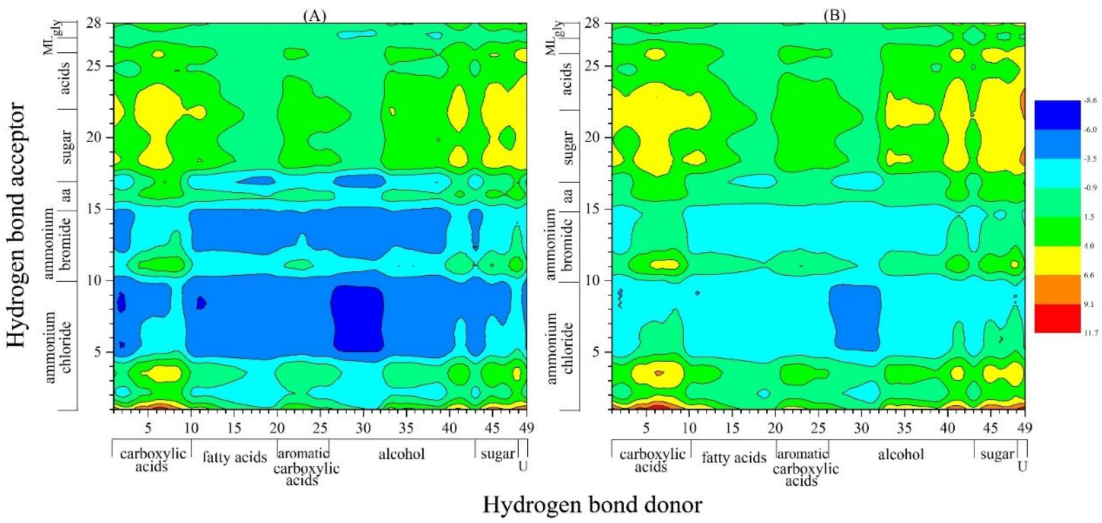

Figure 3. Predicted in DES (1:1) with 30 wt% of water at 35 °C using COSMO-RS **(A)** for carnosic acid; **(B)** carnosol.

The screening of DES by COSMO-RS indicates that hydrophobic DES composed of ammonium chlorides with higher alkyl chain are the best choice to extract carnosic acid and carnosol from rosemary leaves, which leads to extracts with higher antioxidant activity. As such, the selected components for the experimental extraction of antioxidants from rosemary were $N_{2222}Cl$, $N_{3333}Cl$ and $N_{4444}Cl$ HBA and propionic acid, 1,2-propanediol, ethylene glycol, and 5-phenyl-valeric acid as HBD.

Solid-liquid extraction and mixture design

The DES selected in the previous section were prepared with different water contents (10 or 30 wt%) The extraction of carnosic acid and carnosol from

320 rosemary leaves was carried out at 35 °C, during 120 min and using a L-S ratio 321 20:1. Firstly, it was tested the most promising solvents in solid-liquid extractions 322 at a HBA:HBD molar ratio of 1:1. Due to high viscosity and difficulties in obtaining 323 a clear solvent for the pure DES, 1:2 molar ratio proved to be more suitable. The 324 higher ratio of polyols to HBA reduces the surface tension and viscosity of 325 DES.[12,52]

326 The extraction of carnosic acid and carnosol was quantified through antioxidant 327 assays, and the responses obtained are shown in Figure 4. Absent bars in one 328 HBA group indicate that solvent was not stable at those conditions, *e.g.* 329 $N_{2222}Cl$:1,2-propanediol at 10 and 30 wt% of water. Extractions with water, 330 acetone, and ethanol at 70 wt% were used as control.

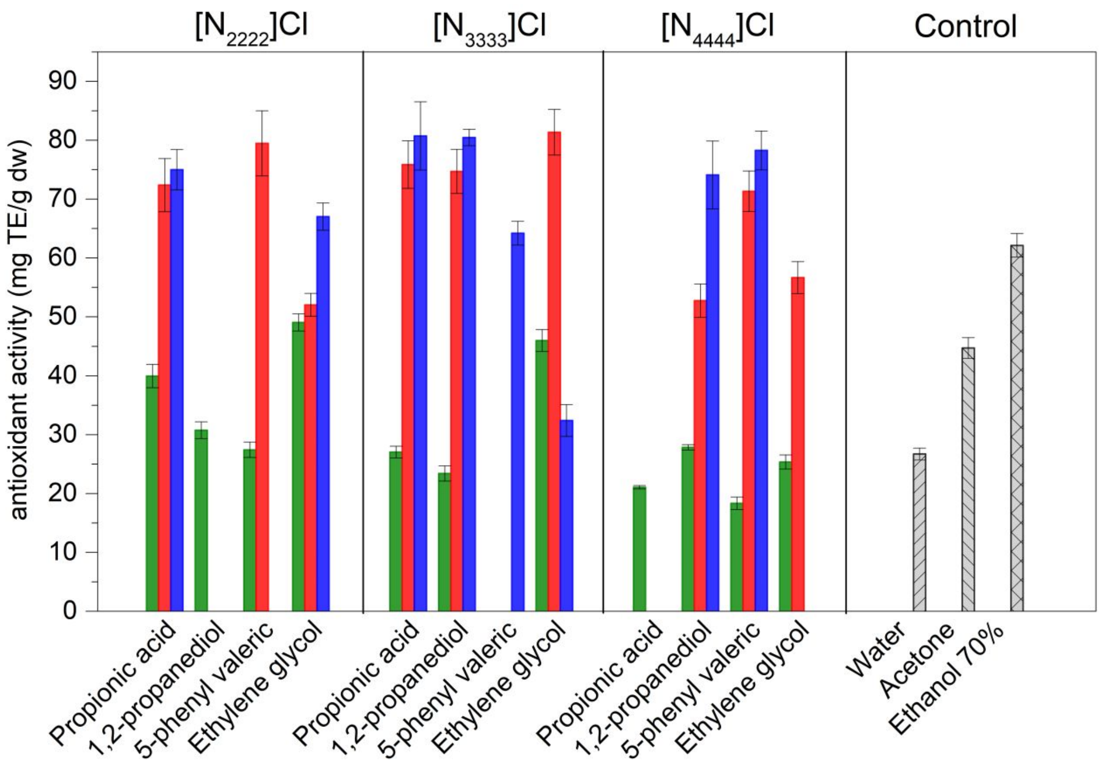

Figure 4. Evaluation of the antioxidant activity of rosemary leaves extracts using different HBA-HBD with the following water contents: 0 wt% green bars; 10 wt% red bars and 30 wt% blue bars. (L-S ratio of 20:1, during 120 min and at 35°C). Grey bars are the controls.

The results for extractions using pure DES showed a performance (low antioxidant activity) similar to water. Nevertheless, in a few cases, *e.g.*, $N_{2222}Cl$: ethylene glycol and $N_{3333}Cl$: ethylene glycol was possible to obtain results comparable to acetone. Note that, unlike predicted by COSMO-RS, the water addition to DES actually increased the antioxidant activity of the extract. Even though water negatively impacts in the solutes solubility, as seen in the COSMO-RS results, it is known that water facilitates the mass transfer and this was determinant to choose the solvent, since water addition improved the extraction efficiency, because of a reduction in the viscosity.[53]Additionally, aqueous solutions of DES composed of $N_{3333}Cl$ as HBA, and propionic acid or 1,2-propanediol as HBD seem to be the more interesting to obtain an extract with high antioxidant activity (≈ 80 mg TE/g dw). It is important to mention that these results were higher than those obtained with water, and the other controls, acetone and ethanol 70 wt%. Controls tests to the antioxidant activity of the solvent used in the extraction were also made, and no significant antioxidant activity was observed.

Mahmood and co-workers[12] pointed the HBA:HBD molar ratio of DES play an important role for extraction efficiency, increasing or decreasing it according to changes in the HBD proportion.[54] Based on it, a ternary mixture design using the most promising HBA, $N_{3333}Cl$, combined to different HBD (propionic acid and 1,2-

propanediol) and water, was made in order to find the best solvent and its composition to obtain an extract with the highest antioxidant activity (Figure 5). The results obtained were analyzed statistically with a confidence level of 95%, and their variance analysis (ANOVA) are shown in the Supporting Information (Figures S5 and S6). No significant differences were observed between the experimental and calculated responses, supporting the good description of the experimental results by the statistical model developed (both analysis present $R^2_{adjusted}$ higher than 0.90). Moreover, the three variables studied (water, HBA and HBD) and their interactions showed to have an impact on the antioxidant activity of the extract according to Pareto charts.

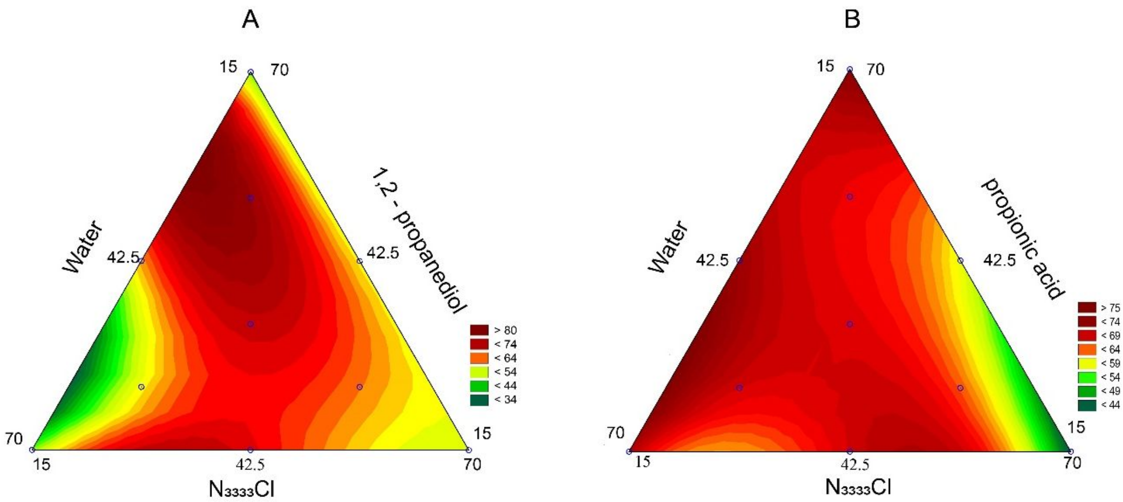

Figure 5. Response surface of antioxidant activity, in mg TE/g dw, as a function of the composition of the ternary (wt %) HBA ($N_{3333}Cl$), HBD (**A**: 1,2-propanediol and **B**: propionic acid) and water, using a L-S ratio of 20:1, during 120 min at 35 °C.

The ternary diagrams in Figure 5 exhibit the antioxidant activity of different solvent compositions, from 15 to 70 wt% of each variable (HBA, HBD and water). The results shown in Figures 5A and 5B, plotting the data reported in Table S6 (Supporting Information), indicated that the extracts obtained with propionic acid as HBD led to lower antioxidant activity values, ≈ 72 mg TE/g dw, when compared to the extract obtained with 1,2-propanediol as HBD, ≈ 77 mg TE/g dw. Moreover, from Figure 5A it is possible to note an enhancement of the antioxidant activity of the extract near the top-left side of the triangle, for water contents below 42.5%. The positive effect of the water results from a decrease in viscosity that facilitates the mass transfer of bioactive solutes to the media. However, excess water became deleterious as it affects the solubility of the hydrophobic antioxidant compounds.[40] Additionally, excessive water can result in the suppression of the interactions between the constituents of the DES. Dai and co-workers observed that structure of the DES Choline chloride: 1,2-propanediol was preserved below 50 wt% of water, and further dilution led to a solution of the free forms of the individual components.[55] In agreement, we observed in this study that a water percentage higher than 42.5% led to a drastic reduction in antioxidant activity. Because of the higher antioxidant performance and also their authorized use by the European Unions as a food additive (E 1520),[56] 1,2-propanediol was chosen as HBD. Thus, only the composition of DES formed by $N_{3333}Cl$: 1,2-propanediol and water was optimized. The results showed that the optimized DES composition that maximized the antioxidant activity of the extract was 15 wt% of

HBA ($N_{3333}Cl$), 55 wt% of HBD (1,2-propanediol) and 30 wt% of water, providing an antioxidant activity of the extract of 79.25 ± 0.91 mg TE/g dw.

Optimization of solid-liquid extraction

A RSM was then used to optimize the operational conditions to obtain an extract with the maximum antioxidant activity. This methodology allows the explanation of the relationship between the response (antioxidant activity, mg of TE/g dw) and the independent variables/conditions which influence the antioxidant activity of the extract (L-S ratio, temperature and time).[57,58] The DES employed to carry this study was composed of 15 wt% of $N_{3333}Cl$, 55 wt% of 1,2-propanediol and 30 wt% of water, taking into account the results obtained in the previous section. The experimental points used in the factorial planning, the antioxidant activity assessed experimentally, as well as all statistical analysis are shown in the Supporting Information (Table S7, Figures S7 and S8). The $R^2_{adjusted}$ value of the polynomial equation for the antioxidant activity was 0.93, showing that no significant differences were observed between the experimental and calculated responses supporting the good description of the experimental results by the statistical models developed.

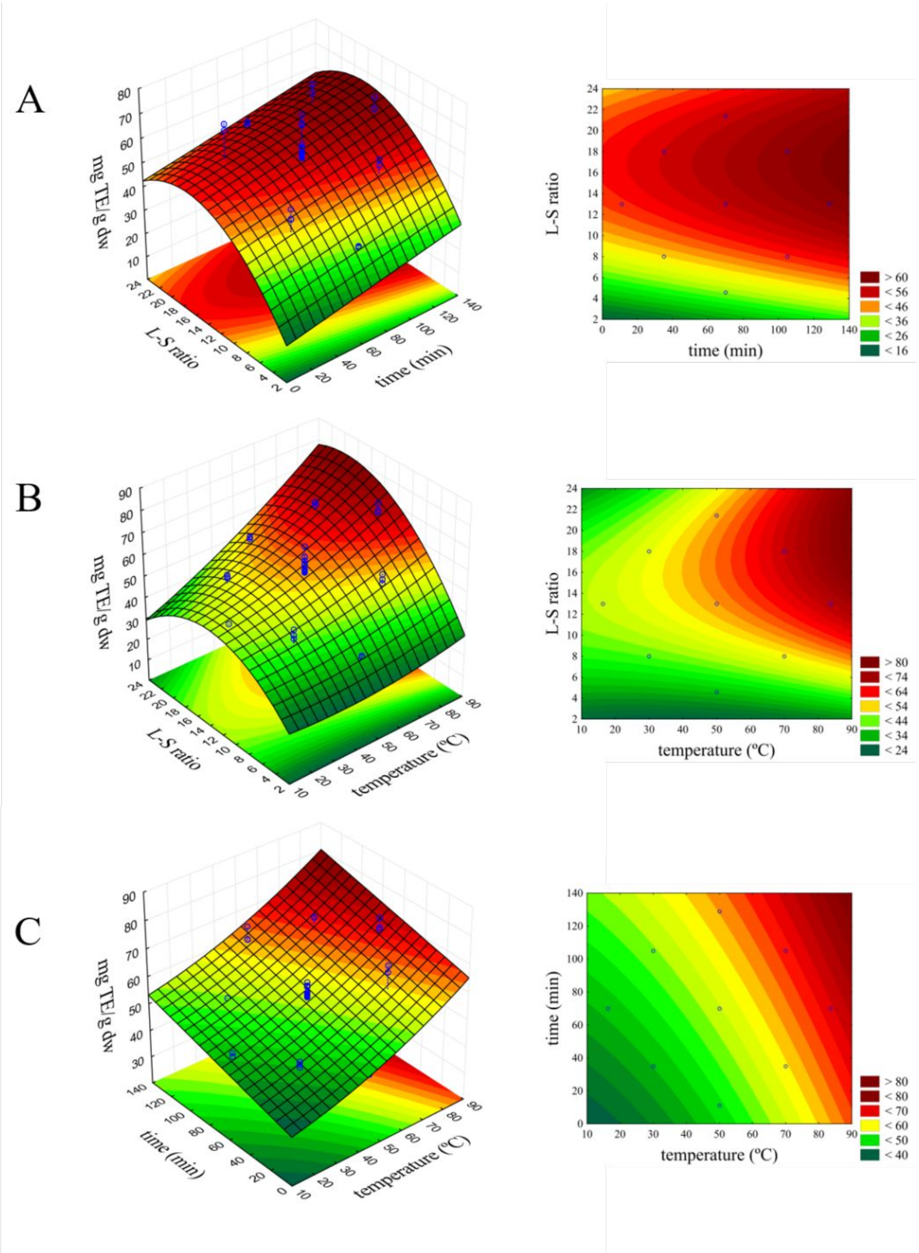

Figure 6. Response surface (left side) and contour plots (right side) of the antioxidant activity using an aqueous solution of DES (15 wt% of $N_{3333}Cl$, 55 wt%

of 1,2-propanediol and 30 wt% of water) with the combined effects: **(A)** S-L ratio and time; **(B)** L-S ratio and temperature; and **(C)** time and temperature.

With the second-order polynomial equation obtained using multi regression analysis, 3D and 2D response surfaces were plotted to express the effects of parameters/variables on the antioxidant activity, Figure 6. It is clear that higher temperatures are more efficient to obtain an extract with higher antioxidant activity. The L-S ratio also has a relevant impact on the antioxidant activity of the extract. Additionally, higher extraction times lead to an enhancement of the response variable, showing that neither the solvent is saturated nor the biomass is depleted in the target compound. Nevertheless, this is the variable with the weakest influence on the antioxidant activity of the extract. Generally, all three parameters, as well as their interactions, have a statistically significant impact on the antioxidant activity of the extract, as can be seen in the Pareto chart (Figure S7, Supporting Information). After regression analysis, the optimized operational conditions that lead to an extract with a high antioxidant activity using the DES with composition optimized (15 wt% of $N_{3333}Cl$, 55 wt% of 1,2-propanediol and 30 wt% of water) occur at a temperature of 84 °C, an extraction time of 129 min, and a L-S ratio of 21:1. At this point, the predicted antioxidant activity was 82.03 ± 3.44 and experimentally it was found 85.04 ± 1.52mg TE/g dw, which demonstrates the predictive ability of the model.

After, all extracts resulting from the RSM were analyzed by HPLC to quantify the amounts of carnosic acid and carnosol present (Table S7, Supporting Information). The lowest extraction efficiency for both compounds, 1.25 mg of carnosic acid and 1.63 mg of carnosol per gram in dry basis, corresponds to the extraction that exhibited the lowest antioxidant activity, 33.8 mg TE/g, *i.e.*, the extraction done with a lower L-S ratio (5:1). In the same line, the highest efficiency, 14.80 mg of carnosic acid/g dw and 18.99 mg of carnosol/g dw corresponds to the extract with higher antioxidant activity, 85 mg TE/ g dw, i.e., the extraction obtained at optimum operational conditions. The extraction efficiency of the two antioxidant compounds for the remaining extractions are also in agreement with their antioxidant activity values (Table S7, Supporting Information). These results show: i) it was possible to get an extract rich in carnosic acid and carnosol; ii) the maximum and minimum values of rosemary phenolics diterpenes extracted correspond to the maximum and minimum values of antioxidant activity.

How does the binary mixture compare with the ternary?

The study of solvent composition using ternary mixtures, led to a parallel discussion besides the statistically one. We demonstrated, by the Pareto chart (Figure S6 Supporting Information I) that the three-independent variable, HBA, HBD and water, were significant. However, as examined at the end of 3.2 section, the optimal solvent composition was obtained with a low concentration in $N_{3333}Cl$. In order to check the importance of HBA presence, we tested binaries mixtures of water and 1,2-propanediol, which can be useful to understand better the potential synergic effects between HBA and HBD.[59]

The conditions of the extraction used were the same as the optimal obtained in section 3.3 (L-S ratio of 21:1, during 129 min), but using two temperatures of the extraction, the optimal one (84 °C), and 35 ºC, used in the mixture design. The selection of these conditions aims at simplifying the extraction process and minimizing the energy consumption by using a simpler solvent, widely applied in the food and pharmaceutical industries,[60] at a lower temperature.

As shown in Figure 7, water addition into 1,2-propanediol has a positive impact, enhancing the antioxidant activity from 46.9 to 65.5 mg TE/g dw, at 35 ºC in agreement with the results reported above. This happens at both temperatures studied. As seen in the ternary mixtures, further water impacted the antioxidant solutes negatively due to the non-polar profiles.[61] There was observed a similar behavior between predicted, by the mixture design, and experimental data of binary mixtures at 35 °C, mainly in the range of 60 to 80 wt% of 1,2-propanediol.

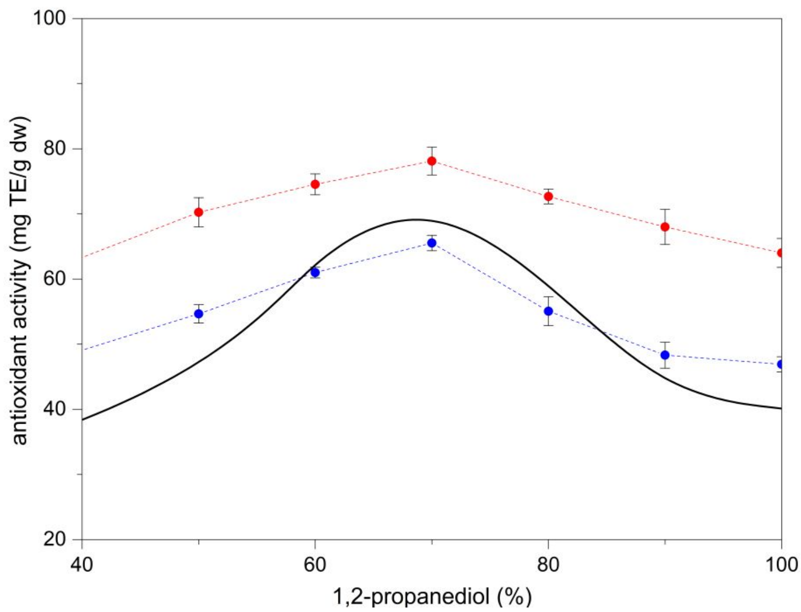

Figure 7. Solid-liquid extraction using a binary mixture of water and 1,2-propanediol (0 – 100 wt%), at 35 °C (blue circles) and 84 °C (red circles), using

a L-S ratio of 21:1 during 129 min. Solid black line is the predicted data by the mixture design at 35°C. Dashed lines provide a guide for the eye.

Moreover, comparing the values obtained for the two temperatures in studied (84 °C and 35 °C), the highest antioxidant activity values were obtained for the highest temperature of extraction (84 °C). As found in the RSM, temperature exhibits a positive effect to enhance the antioxidant activity of rosemary extracts. Note that the use of these temperatures is not a problem for the antioxidant molecules in the study (carnosic acid and carnosol) as they are temperature stable and it was shown that at 170 ºC these phenolic diterpenes still present antioxidant activity.[62]

The results reported show that the binary mixtures (water + 1,2-propanediol) may be a good alternative to extract this class of bioactive compounds from rosemary leaves, leading to better results than water and the other control solvents studied. For the extraction that occurs at 84 °C, during 129 min and a L-S ratio of 21:1, using a binary mixture with 70 wt% of 1,2-propanediol and 30 wt% of water was possible to obtain an antioxidant activity of 78.12 mg TE/g dw.

This value was lower than the one obtained at the optimal operational conditions using the ternary DES mixture (85.04 ± 1.52mg TE/g dw). Mahmood and co-workers[12,54] also obtained better extraction efficiencies of phenolics when they used polyol-based DES than when they used only their individual components since, DES may favor electrostatic and hydrogen bond interaction with the target solutes, leading to higher extraction efficiencies.[12,54] Nevertheless, the absence of HBA may compensate in simplicity, the loss in performance. Since 1,2 propanediol is a compound that can be evaporated it would allow the recovery of the antioxidant extract by evaporation, or, depending on the application, since it is a compound approved as food additive it would be possible to use the extract directly as final product.

CONCLUSION

Natural antioxidants could replace synthetic ones, and for this, it is necessary to identify appropriate solvents that allow to solubilize these solutes. COSMO-RS was used as a screening tool on the choice of solvents in the extraction of carnosic acid and carnosol from rosemary leaves. It is shown that COSMO-RS allows a quick and qualitative *in silico* evaluation of the solubility of bioactive molecules in a large number of solvents, and consequently reduce the number of solvents to be tested at the laboratory. This computational experiment revealed ammonium chlorides, and the fatty acids, aromatic carboxylic acids, or alcohols to be the best HBA and HBD, respectively. By an experimental design, the optimal solvent composition was established to be 15 wt% of $N_{3333}Cl$, 55 wt% of 1,2-propanediol and 30 wt% of water (79.25 ± 0.91 mg TE/g dw). The conditions of extraction were further optimized by RSM showing that the extractions made 84 ºC, during 129 min and L-S ratio of 21:1, can lead to the highest antioxidant activity of rosemary extract, 85.04 ± 1.52 mg TE/g dw. Furthermore, the HPLC results were in agreement with the antioxidant activity data, *i.e.,* the extracts richer in carnosol and carnosic acid were the ones that present a higher antioxidant activity. Despite the optimal result obtained from DES, aqueous solutions of 1,2-propanediol (70 wt%) were shown to be a good alternative to the ternary DES due to the simplicity that it would impart to the recovery of the solvent or adopting a strategy of leave-in.

Acknowledgments

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. J.P. Wojeicchowski is grateful for the scholarship (88881.361904/2019-01) provided by CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil*) and by Banco Santander S.A (Brazil). M. R. Mafra is grateful to the Brazilian National Council for Scientific and Technological Development (CNPq - Grant 308517/2018-0).

Supporting information:

The Supporting Information is available free of charge on the ACS Publications website at DOI: XX.XXX/XXXXXXX.

The hydrogen bond acceptor list used in the COSMO-RS prediction; the hydrogen bond donor list used in the COSMO-RS prediction; the table of mixture design for solvent composition optimization; the factorial planning used by response surface methodology; the coded levels of independent variables; the contour map of predicted activity coefficient of carnosic acid with no water; the contour map of predicted activity coefficient of carnosic acid with 30 wt% of water added; the contour map of predicted activity coefficient of carnosol with no water; the contour map of predicted activity coefficient of carnosol with 30 wt% of water added; the Pareto charts for the mixture design using propionic acid as hydrogen bond donor; the Pareto charts for the mixture design using 1.2-propanediol as hydrogen bond donor; the antioxidant activity data obtained by the mixture design; the antioxidant activity data and the HPLC results obtained from central composite design; the Pareto chart for the central composite design for the antioxidant activity of the extract, and the predicted *vs* observed values of antioxidant activity.

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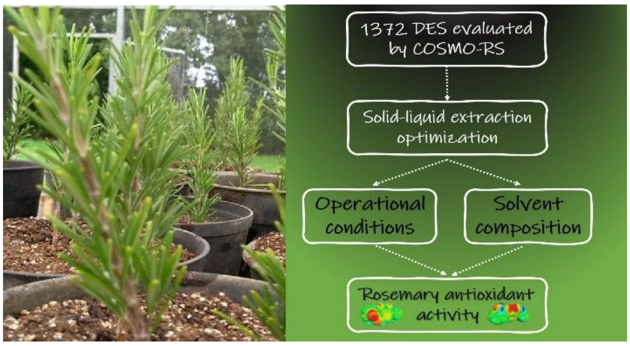

Synopsis: Solvent and operational conditions were both optimized to enhance antioxidant activity from rosemary extract using a thermodynamic predictive model and experimental procedures.