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Combination of Lactic Acid-Based Deep Eutectic Solvents (DES) with β-Cyclodextrin: Performance Screening Using Ultrasound-Assisted Extraction of Polyphenols from Selected Native Greek Medicinal Plants

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Abstract: A series of novel L-lactic acid-based deep eutectic solvents (DES) were tested for polyphenol extraction performance, using organically grown, native Greek medicinal plants. The extractions were ultrasonically-assisted and the effect of the addition of β-cyclodextrin (β-CD) as extraction booster was also tested, at a concentration of 1.5% (*w*/*v*). The estimation of total polyphenol yield (Y_{TP}) suggested that DES composed of L-lactic acid and nicotinamide and L-lactic acid and L-alanine, both at a molar ratio of 7:1, are promising solvents giving significantly higher yields compared with 60% (*v*/*v*) aqueous ethanol and water. However, when β-CD was combined with DES comprised of L-lactic acid and ammonium acetate (molar ratio 7:1), the extraction yields obtained in some instances were equal of even higher. The pattern was not consistent when the yield in total flavonoids (Y_{TFn}) was considered, indicating water, 60% (*v*/*v*) aqueous ethanol and L-lactic acid:sodium acetate (molar ratio 7:1) to be the most efficient solvents. In this case, the effect of β-CD was of rather lower magnitude. The examination of the antioxidant activity of the extracts generated showed that there is a close correlation mainly with their concentration in total polyphenols.

Keywords: antioxidants; β-cyclodextrin; deep eutectic solvents; medicinal plants; polyphenols; ultrasound-assisted extraction

1. Introduction

The use of medicinal plants to sustain a good health status and to reduce disorders and degenerative diseases has been recognised since antiquity and to date they offer a potential advantage because they comprise of an array of components with multiple beneficial effects. However, the quality and content of the active supplement depends on collection, processing and composition of the raw material and extraction procedures [1]. Herbal preparations are broadly used as medicines and nutritional supplements, and the share of herbal medicines is estimated to be about US$60 bn, having an annual growth rate of 5–15%; this makes herbal drugs a significant part of the total world pharmaceutical market [2].

The pharmaceutical potantial of numerous herbal formulations are frequently ascribed to polyphenolic compounds [3], which have attracted a great attention because of their multifaceted biological activities [4], including antioxidant activity, antimicrobial activity, as well as chemoprotective potency. For this reason, there have been a number of investigations on the exploitation of

polyphenol-containing medicinal plants. The interest is on the development of highly performing and cost-effective downstream processes, which aim at producing commodities with crude or purified extracts.

A critical parameter in such processes is the efficient retrieval of polyphenols, which is primarily carried out by solid-liquid extraction. The selection of an appropriate solvent is of paramount importance and profoundly defines yield and composition of the extracts produced. Most common solvents to date are of petrochemical origin [5] but they have several inherent disadvantages, being flammable and toxic, while their production is associated with fossil resources. A high number of examinations have been expended on the replacement of hazardous solvents with more eco-friendly alternatives, and in this regard biomass-derived materials offer unprecedented opportunities for green solvent production.

Deep eutectic solvents (DES) are an innovative class of eco-friendly liquids comprised of bio-molecules, such as a polyol serving as the hydrogen bond donor (HBD), and an organic salt, which is the hydrogen bond acceptor (HBA) [6]. DES display attractive attributes, including negligible vapour pressure, tunability, lack of toxicity, low cost, etc. These features make DES ideal extraction solvents, possessing unique characteristics, whereas limitations associated with similar materials, such as conventional organic solvents and ionic liquids, may be overcome [7]. DES are usually less expensive, easy to produce (use of bulk commodity chemicals) and biodegradable and, depending on the composition, they may be compatible with foods, pharmaceuticals and cosmetics. Furthermore, tailoring their physicochemical properties could be crucial to manipulating their extraction potency, because several parameters (polarity, viscosity, hydrogen bonding) central to mass transport phenomena may be effectively regulated [8].

Cyclodextrins (CDs) are enzymicaly produced starch derivatives, and owed to the slightly hydrophobic inner surface of their ring-shaped molecules, they can form inclusion complexes with molecules of low hydrophilicity and suitable geometrical size. These cyclic oligosaccharides comprise of 6, 7 or 8 glucopyranose units (α-, β- and γ-CDs, respectively) and they differ in size (0.5–0.9 nm in diameter) that determines the accommodation of the guest molecules into the cavity [9]. Recent examinations showed that combining 2-hydroxypropyl β-cyclodextrin with aqueous glycerol may result in increased yield of polyphenol extraction [10,11]. Similar outcome was reported for water extraction of apple flavonols, using various cyclodextrins [12]. In this framework, this investigation was undertaken with the aim to screening the performance of polyphenol extraction from various organically grown native Greek medicinal plants, using a combination of β-CD with a series of L-lactic acid-based DES.

2. Experimental Section

2.1. Chemicals

β-Cyclodextrin (98%) and ethanol (99.8%) were from Acros Organics (Geel, Belgium). Anhydrous sodium carbonate was from Carlo Erba Reactifs (Val de Reuil, France). Nicotinamide was from Fluorochem (Hadfield, UK). L-Alanine (99%) and choline chloride (99%) were from Alfa Aesar (Karlsruhe, Germany). Aluminium chloride hexahydrate, ammonium acetate, sodium acetate trihydrate, and L-lactic acid (80%) were from Penta (Prague, Czeck Republic). Glycine (99.5%) was from NeoLab Migge Laborbedarf-Vertiebs (Heildelberg, Germany). 2,4,6-Tripyridyl-*s*-triazine (TPTZ, 99%), Folin-Ciocalteu reagent and ferric chloride hexahydrate were from Fluka (Steinheim, Germany). 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), caffeic acid, ascorbic acid and rutin (quercetin 3-*O*-rutinoside) were from Aldrich (Steinheim, Germany).

2.2. Preparation of DES

Previously, reported methodologies were applied to synthesise all the DES tested [13]. Briefly, HBD (L-lactic acid) was mixed with each of the HBAs (Figure 1), in 100-mL glass vials, at HBD:HBA

molar ratio ($R_{mol}^{D/A}$) of 7:1. Mixtures were heated at 80–90 °C for 25–35 min, under continuous stirring on a magnetic stirrer, at 400 rpm, until perfectly transparent liquids were formed. The liquids were stored in sealed glass containers in a dark chamber, at room temperature (22 ± 2 °C) and were visually inspected for the development of crystals over a period of 10 days. The codes of all DES are presented in Table 1.

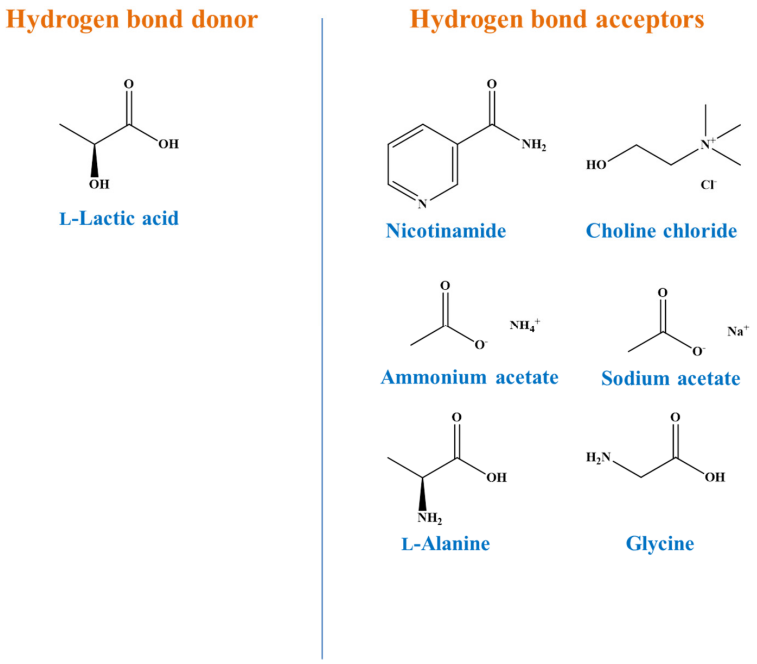

Figure 1. Formulae of L-lactic acid (HBD) and the HBAs used in this study.

Table 1. Codes and names of the medicinal plant specimens used in this study.

Code	Common Name	Systematic Name
CC	Thyme	*Coridothymus capitatus*
OV	Oregano	*Origanum vulgare hirtum*
SF	Greek sage	*Salvia fruticosa (triloba)*
SO	Sage	*Salvia officinalis*
TV	Thyme	*Thymus vulgaris*

2.3. Plant Material

The medicinal plants studied belong to the Lamiaceae family, they are native to the island of Lemnos (Northern Greece) and represent some of the most popular species used in Greece as condiments and folk remedies. All specimens were certified and provided by the AegeanOrganics Ltd. The plants were organically grown under identical conditions of soil, watering and sun exposure and delivered in dried form, in plastic packaging. Upon receipt, the plant material was milled in a table mill (Tristar, Tilburg, The Netherlands) to give powder with average particle diameter of approximately 0.5 mm. The milled material was placed in plastic, sealed containers and stored in a dry and dark chamber for no longer than 7 days.

2.4. Batch Ultrasound-Assisted Extraction (UAE)

Amount of 0.57 g of dried and pulverized plant material was introduced into a 26-mL screw-cap glass vial (diameter = 2.7 cm, height = 4.5 cm), with 20 mL of solvent to give a liquid-to-solid ratio ($R_{L/S}$) of 35 mL g^{-1}. In the cases where β-CD was used, it was incorporated to the mixture at a final

concentration of 1.5% (*w*/*v*). The mixture was subjected to UAE in a temperature-controlled sonication bath (Elma P70, Singen, Germany), with frequency set at 37 Hz, power at 140 W and acoustic energy density at 38.9 W L^{-1}. Extractions were carried out for 60 min, at 55 °C. For the extractions, 75% (*w*/*v*) aqueous DES solutions were employed, along with 60% (*v*/*v*) aqueous ethanol and distilled water, which served as control solvents.

2.5. Sample Preparation and Determinations

After the completion of the extraction, an aliquot of 1 mL of each extract was placed in 1.5-mL Eppendorf tube and centrifuged in a table centrifugator (Hermle, Wehingen, Germany), at 20,000× *g* for 10 min. All samples were diluted 1:20 with methanol prior to determinations. Yield in total polyphenol (Y_{TP}) was determined with the Folin-Ciocalteu methodology, as described elsewhere [14]. Yield in total flavonoids (Y_{TFn}) was determined according to a previously published protocol [15]. The antiradical activity (A_{AR}) and reducing power (P_R) were determined with well-established methods [16].

2.6. Statistical Analysis

Extractions were performed twice and all determinations were performed in triplicate. Results were given as average values ± standard deviation. Value distributions and pairwise correlations were calculated by carrying out distribution analysis and multivariate analysis, respectively, at least at a 95% significance level ($p < 0.05$). For all statistics, Microsoft™ Excel and JMP™ 10 were used.

3. Results and Discussion

3.1. DES Synthesis

The evidence accumulated so far has shown that extraction of polyphenolic compounds from various plant sources using DES may depend significantly on the nature of DES constituents. However, recent studies on polyphenol recovery from a red grape pomace suggested L-lactic acid-based DES to be far more efficient than those containing glycerol or 2,3-butanediol as the HBD [17]. On the other hand, an extensive screening of L-lactic acid-based DES with different amino-bearing HBAs indicated that the nature of HBA also plays a prominent role in the polyphenol extraction efficiency [18]. This being the case, the investigation undertaken aimed at synthesising L-lactic acid-based DES with highly efficient HBAs, including two bases (choline chloride and nicotinamide), two salts (sodium and ammonium acetate) and two amino acids (L-alanine and glycine) (Figure 1).

Judgement upon the potency of the HBAs selected was based on evidence provided by the polyphenol extraction from a few agri-food wastes [19], botanicals [13] and industrial cereal solid wastes [18]. Thus, efforts were made to compose stable DES, by combining the HBD and HBAs at appropriate molar ratios ($R_{mol}^{D/A}$). Although HBAs such as sodium acetate, ammonium acetate and choline chloride may readily produce stable DES at $R_{mol}^{D/A}$ of 1:1, L-alanine and glycine interacted with the HBD at $R_{mol}^{D/A}$ higher than 6:1. To maintain identical composition, all the DES were produced using $R_{mol}^{D/A}$ of 7:1 (Table 2). It is to be emphasised that the DES coded as LA-Nic, to the best of the authors' knowledge, is reported for the first time.

Table 2. Codes of the DES synthesised and the corresponding HBAs used. All DES were tested at $R_{mol}^{D/A}$ of 7:1.

Code	HBA	Appearance
LA-Nic	Nicotinamide	Colourless
LA-Ccl	Choline chloride	Colourless
LA-Sac	Sodium acetate	Colourless
LA-Aac	Ammonium acetate	Colourless
LA-Gly	Glycine	Colourless
LA-Ala	L-Alanine	Colourless

3.2. Extraction Efficiency

The screening process was designed to select highly effective DES and for this purpose a simple and efficient extraction methodology was implemented, to enable simultaneous extraction of a large number of samples. The liquid-to-solid ratio ($R_{L/S}$) was set at 35 mL g^{-1}, which was an average level within the optimum range of 29.5 to 36.2 mL g^{-1} found for polyphenol extraction from *Satureja thymbra* with DES [20]. Extraction temperature was maintained at 55 °C, because higher temperatures may not favour increased polyphenol yield and extraction time was 60 min, which according to kinetic investigations, appeared sufficiently long to prevent variances that could stem from incomplete extraction [21]. The extraction was assisted by ultrasonication, which has been shown to promote higher extraction yields when viscous solvents are employed at moderate temperatures [22].

Two eco-friendly solvents conventionally used for polyphenol extraction were tested as reference solvents, including water and 60% (*v*/*v*) aqueous ethanol. All DES were tested as 75% (*w*/*v*) aqueous solutions, using an average water content of those previously reported for polyphenol extraction with DES [21,23]. Appropriate amounts of water are considered critical for DES extraction efficiency, given the usually high DES viscosity. Water regulates effectively DES viscosity downwards, thus facilitating diffusion-controlled extraction. Moreover, water also modifies DES polarity, which in several instances has been shown to play a key role in solute (polyphenol) solubility, entailing higher extraction yield [20].

The results of the performance screening with regard to total polyphenol yield are given in Table 3. It was observed that in several cases DES were far more effective than water and significantly so than aqueous ethanol ($p < 0.05$). Although no particular pattern was recorded regarding composition-efficiency relationships, it was noticed that LA-Alan gave statistically higher values for *Coridothymus capitatus* (CC) and *Thymus vulgaris* (TV) extraction, LA-Niam for CC, *Salvia fruticosa* (SF) and *Salvia officinalis* (SO), while LA-AmAc and LA-ChCl performed exceptionally only for *Origanum vulgare*. In no case LA-Glyc and LA-SoAc gave statistically higher Y_{TP}. The richest extract was obtained from OV using LA-ChCl (140.72 mg CAE g^{-1} dw), while the lowest performance was seen for the extraction of CC with water, which yielded 33.91 mg CAE g^{-1} dw. The OV yield is similar to 151 mg CAE g^{-1} dw previously reported [24], yet as high as 288 mg sinapic acid equivalents per g dw have been found [25].

Table 3. Y_{TP} (mg CAE g^{-1} dw) of the extracts obtained using DES and β-CD/DES combination. Asterisk denotes statistically higher value ($p < 0.05$).

Solvent	Y_{TP} (mg CAE g^{-1} dw)				
	CC	**OV**	**SF**	**SO**	**TV**
Water	33.91 ± 3.05	107.18 ± 9.65	34.87 ± 3.27	78.10 ± 7.03	50.02 ± 4.34
60% EtOH	63.17 ± 5.69	124.61 ± 11.21	55.09 ± 4.96	96.77 ± 8.71	73.98 ± 6.66
LA-Alan	69.92 ± 6.29 *	129.82 ± 9.69	65.51 ± 5.90	75.84 ± 6.83	97.29 ± 8.67 *
LA-AmAc	57.37 ± 5.16	134.80 ± 12.13 *	51.46 ± 4.63	92.38 ± 8.31	69.60 ± 6.26
LA-ChCl	61.51 ± 5.54	140.72 ± 12.66 *	62.97 ± 5.67	92.73 ± 8.35	88.73 ± 7.99
LA-Glyc	60.48 ± 5.44	102.62 ± 7.20	65.17 ± 5.86	90.72 ± 7.16	83.83 ± 7.54
LA-Niam	72.45 ± 6.52 *	130.60 ± 9.75	73.54 ± 6.62 *	102.70 ± 7.98 *	87.28 ± 7.86
LA-SoAc	66.32 ± 5.97	121.57 ± 8.94	58.72 ± 5.28	98.60 ± 7.88	81.53 ± 5.34
with 1.5% (*w*/*v*) β-CD					
LA-Alan	60.99 ± 5.49	104.37 ± 9.39	66.51 ± 4.99 *	108.94 ± 7.80 *	88.32 ± 7.95
LA-AmAc	72.48 ± 6.52 *	117.50 ± 5.57	62.03 ± 5.58	101.12 ± 9.10 *	102.99 ± 9.27 *
LA-ChCl	59.03 ± 5.31	131.60 ± 11.84	62.55 ± 5.63	93.35 ± 8.40	95.42 ± 8.59 *
LA-Glyc	58.92 ± 5.30	113.58 ± 10.22	59.55 ± 3.36	97.81 ± 6.80	83.89 ± 6.45
LA-Niam	66.54 ± 3.98	144.92 ± 10.04 *	69.02 ± 3.21 *	110.88 ± 5.88 *	27.66 ± 2.49
LA-SoAc	63.18 ± 5.69	124.08 ± 11.17	62.97 ± 5.35	87.59 ± 7.88	79.00 ± 6.11

When Y_{TFn} was considered as extraction performance indicator, the overall picture was profoundly different (Table 4). In all cases examined aqueous extracts, as well as extracts obtained with aqueous ethanol were shown to give statistically higher values ($p < 0.05$). LA-SoAc was the most efficient solvent, providing significantly higher Y_{TFn} for CC, SF and TV, while LA-Niam performed likewise for OV. By contrast, LA-Alan, LA-AmAc, LA-ChCl and LA-Glyc did not show increased efficacy for any of the specimens tested. The highest Y_{TFn} was found for the SF extract obtained with aqueous ethanol, amounting 30.89 mg RtE g^{-1} dw, but the aqueous ethanolic extract from SO had comparable Y_{TFn} of 28.93 mg RtE g^{-1} dw. Total flavonoid content in SO extracts was determined to be 16.89 mg quercetin equivalents per g dw [26], but levels as low as 3.50 mg RtE g^{-1} dw have also been found [27].

Table 4. Y_{TFn} (mg CAE g^{-1} dw) of the extracts obtained using DES and β-CD/DES combination. Asterisk denotes statistically higher value ($p < 0.05$).

Solvent	Y_{TFn} (mg RtE g^{-1} dw)				
	CC	**OV**	**SF**	**SO**	**TV**
Water	9.64 ± 0.39 *	21.13 ± 0.85 *	22.46 ± 0.90 *	25.96 ± 1.04 *	20.46 ± 0.98 *
60% EtOH	10.81 ± 0.43 *	23.89 ± 0.96 *	30.89 ± 1.24 *	28.93 ± 1.16 *	28.94 ± 0.87 *
LA-Alan	6.78 ± 0.37	14.26 ± 0.57	13.48 ± 0.54	14.46 ± 0.58	18.19 ± 0.95
LA-AmAc	5.43 ± 0.32	15.80 ± 0.33	13.03 ± 0.52	16.07 ± 0.84	14.07 ± 0.43
LA-ChCl	4.93 ± 0.20	12.83 ± 0.61	14.76 ± 0.87	13.48 ± 0.54	15.61 ± 0.62
LA-Glyc	6.39 ± 0.46	9.03 ± 0.38	15.45 ± 0.62	12.30 ± 0.59	10.87 ± 0.76
LA-Niam	7.51 ± 0.32	20.54 ± 0.90 *	17.58 ± 0.70	18.83 ± 0.75	16.91 ± 0.68
LA-SoAc	10.94 ± 0.44 *	15.80 ± 0.97	20.57 ± 1.02 *	21.13 ± 1.02	20.35 ± 0.81 *
with 1.5% (*w*/*v*) β-CD					
LA-Alan	3.27 ± 0.13	12.36 ± 0.49	17.05 ± 0.76	18.86 ± 0.75	17.95 ± 0.71
LA-AmAc	5.43 ± 0.21	14.02 ± 0.56	17.17 ± 0.99	18.80 ± 0.62	21.68 ± 0.77 *
LA-ChCl	3.43 ± 0.14	12.41 ± 0.50	14.19 ± 0.57	14.29 ± 0.57	13.62 ± 0.76
LA-Glyc	4.40 ± 0.18	10.37 ± 0.76	12.11 ± 0.68	13.11 ± 0.52	12.41 ± 0.50
LA-Niam	9.25 ± 0.37 *	15.05 ± 0.60	19.38 ± 0.78	21.87 ± 0.99 *	6.03 ± 0.24
LA-SoAc	8.68 ± 0.45 *	19.38 ± 0.78 *	19.13 ± 1.00	18.58 ± 0.74	20.87 ± 0.98 *

3.3. The Effect of β-Cyclodextrin (β-CD) Addition

Polyphenol extraction with the aid of β-CD has been shown to affect both extraction yield and extract composition, due to selectivity of β-CD towards complexation of certain polyphenols [28,29]. Aqueous solutions of β-CD have been successfully used to assist polyphenol extraction from various matrices, including pomegranate [30], tea leaves [31], vine shoots [32] and *Polygonum cuspidatum* [33], but combination of β-CD with DES has never been reported. In the view of clarifying whether such a combination could boost polyphenol extraction yield, extractions with the DES tested were also performed in the presence of 1.5% (*w*/*v*) β-CD. The amount of β-CD was carefully chosen, in the light of previous data [30,33].

The addition of β-CD changed drastically the overall picture of total polyphenol yield obtained from the extractions carried out only with DES (Table 3). For CC, β-CD increased the extraction capacity of LA-AmAc by approximately 21%, which gave significantly higher Y_{TP} ($p < 0.05$). On the contrary, LA-Alan and LA-Niam, which exhibited high performance in the absence of β-CD, showed lower efficacy. Similarly, β-CD boosted total polyphenol recovery from OV with LA-Niam, but depressed the efficiency of LA-AmAc and LA-ChCl. For the SF extraction, β-CD incorporation improved the capacity of LA-Alan and for SO improvement was seen with LA-Alan and LA-AmAc. For TV extraction, LA-Alan efficiency dropped significantly upon addition of β-CD ($p < 0.05$), but LA-AmAc displayed over than 32% higher Y_{TP}. Regarding Y_{TFn}, β-CD enhanced LA-Niam performance for CC and SO, LA-AmAc for TV and LA-SoAc for OV, but depressed extraction of SF with LA-SoAc (Table 4). Overall, TFn recovery was impacted to a rather lesser extent compared with TP.

Encapsulation of a ligand in the cavity of CD obeys a certain stoichiometry, which is usually 1:1 [34]. In aqueous media the displacement of water outside the β-CD cavity and retention of the polyphenol within through hydrophobic association is the driving force for polyphenol-β-CD complex formation [35]. However, polyphenols may behave as HBDs and interact with the HBA of the DES, which may give rise to some sort of antagonism, because β-CD might weaken polyphenol-HBA interactions since polyphenol/β-CD complex formation involves strong hydrogen bond formation [36]. On the other hand, β-CD would increase solubility of the less polar polyphenols through entrapment. Therefore, the eventual outcome could represent the integration of such an effect.

3.4. Antioxidant Activity

Two representative and complementary tests were carried out to assess the antioxidant activity of all the extracts generated. With the exception of SF extracts, significantly higher A_{AR} levels were determined for those obtained with LA-AmAc/β-CD. The same phenomenon was observed for LA-Alan/β-CD, with the exception of OV (Figure 2). This finding highlighted the importance of these extraction media in obtaining extracts with strong antiradical effects. It should also be stressed that in the cases of CC, SF and TV, LA-Alan was also very effective in providing extracts with significantly higher A_{AR} ($p < 0.05$). By contrast, exceptionally high P_R was displayed mainly by the extracts obtained with aqueous ethanol (Figure 3), and no consistency with A_{AR} was found.

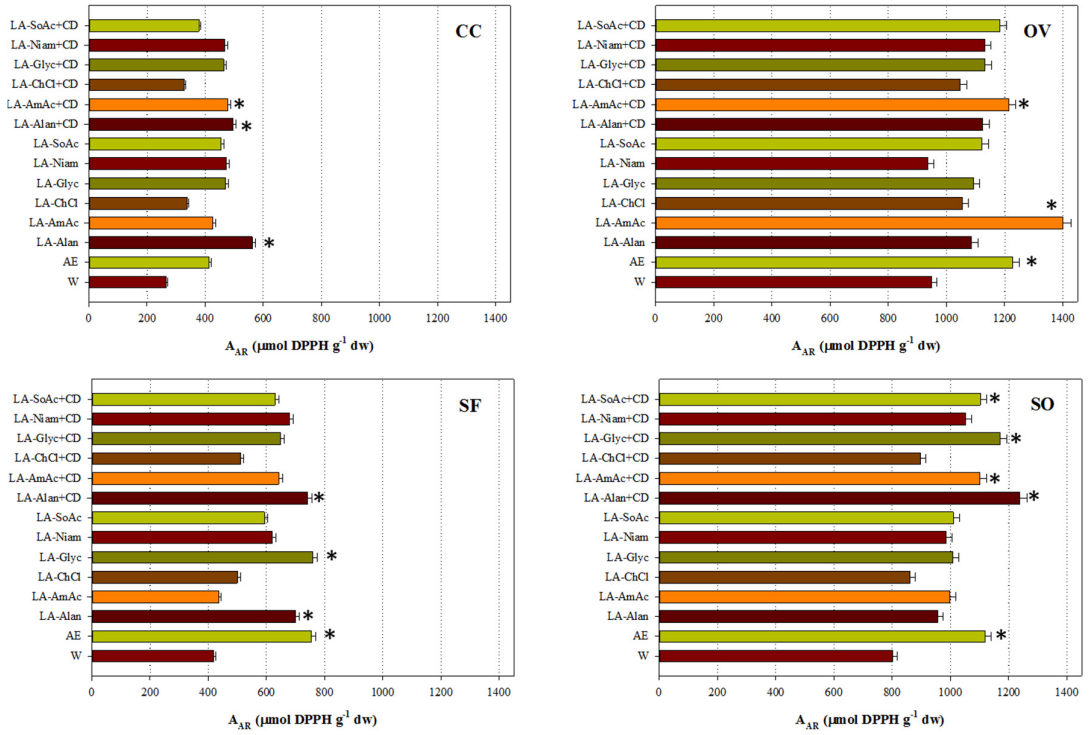

Figure 2. *Cont.*

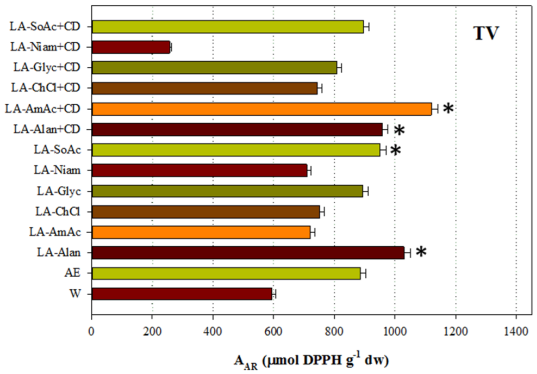

Figure 2. Plot showing the A_{AR} of the extracts obtained. Asterisk denotes statistically higher value ($p < 0.05$). Assignments: CC, *Coridothymus capitatus*; OV, *Origanum vulgare*; SF, *Salvia fruticosa*; SO, *Salvia officinals*; TV, *Thymus vulgaris*.

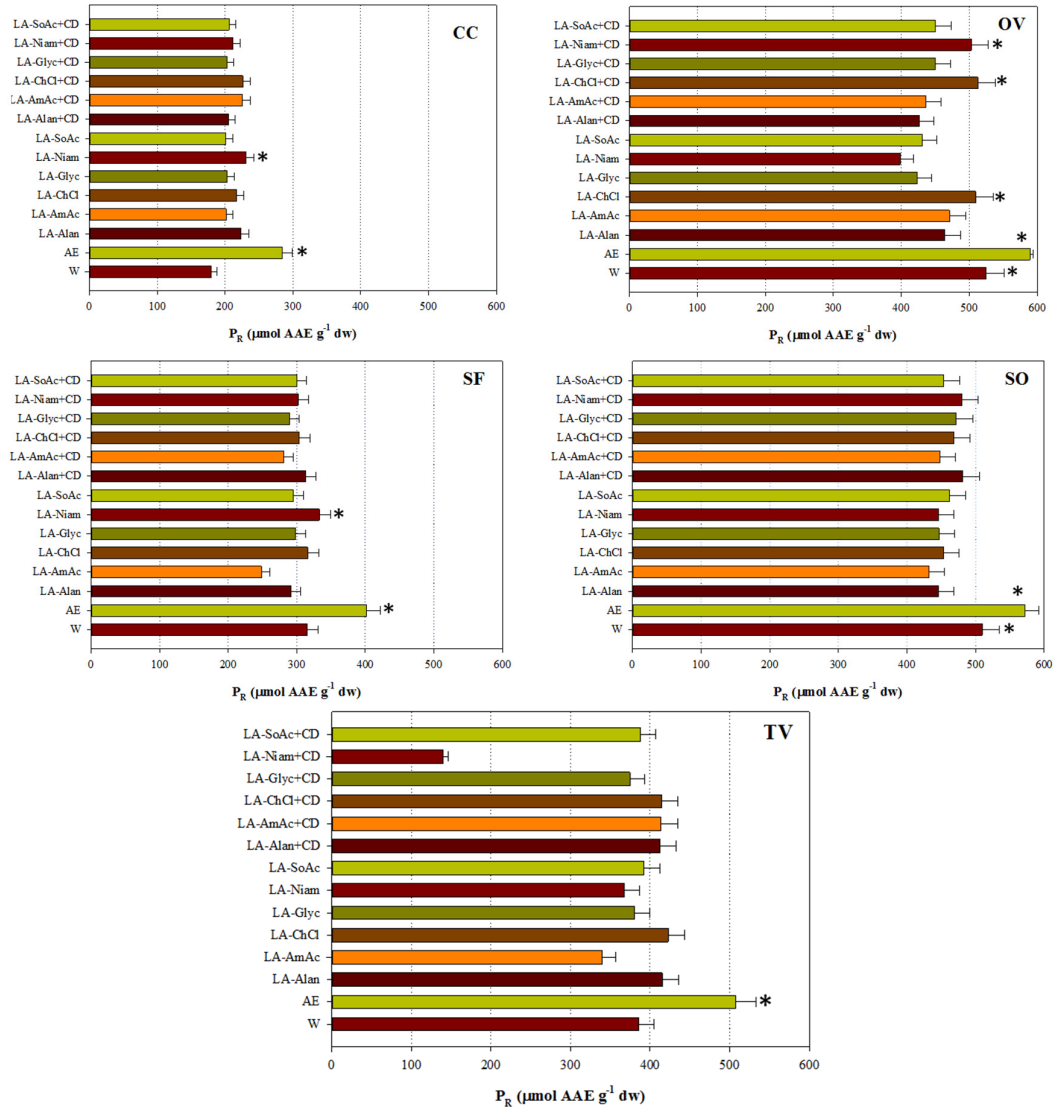

Figure 3. Plot showing the P_R of the extracts obtained. Asterisk denotes statistically higher value ($p < 0.05$). Assignments: CC, *Coridothymus capitatus*; OV, *Origanum vulgare*; SF, *Salvia fruticosa*; SO, *Salvia officinals*; TV, *Thymus vulgaris*.

The role of β-CD in the expression of antioxidant effects should not be overlooked, because it has been demonstrated that β-CD complexes with rosmarinic acid [37], quercetin and rutin [38], but also hydroxypropyl β-CD complexes with polyphenols such as chlorogenic acid [39] and quercetin [36], showed significantly higher antioxidant activity compared with the non-encapsulated polyphenols. This phenomenon has also been demonstrated for chlorogenate-rich coffee extracts [40]. The orientation of the encapsulated molecule in the β-CD cavity is probably critical in this respect. It has been shown that complex of β-CD with oleuropein from olive leaf extracts involves deep insertion of the dihydroxyphenethyl moiety inside the cavity from its secondary side [41]. This could affect oxidant/antioxidant interactions and therefore, the antioxidant effects observed in DES/β-CD extracts may not be simply attributed to higher polyphenol concentration, but to interactions associated with polyphenol/β-CD complexes as well.

In Figure 4 it can be seen that the correlation of Y_{TP} with both P_R and A_{AR} was highly significant ($p < 0.0001$) and the same held true for Y_{TFn} ($p < 0.0001$). However, R^2 was notably higher for correlations with Y_{TP}. This outcome highlighted the importance of total polyphenol concentration for the antioxidant activity of the extracts. Similar outcome was seen for various agri-food waste extracts [19], but also botanicals [13]. Nevertheless, mixture effects that can be manifested either as synergism or antagonism among the various polyphenols occurring in the extracts should also be considered. Such phenomena were demonstrated for A_{AR} in mixtures of antioxidants [42] and mixtures of antioxidants with plant extracts [43]. Similar mixture effects for P_R have also been found [44].

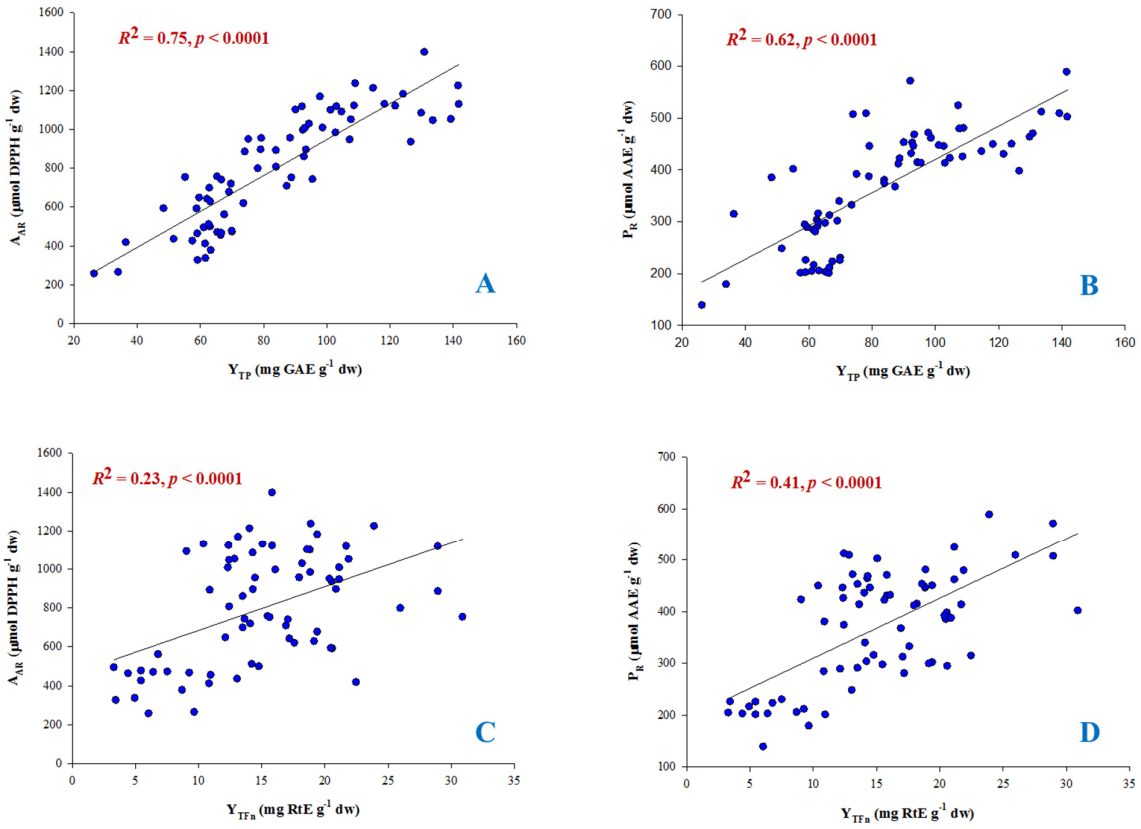

Figure 4. Plots illustrating linear regression between (**A**) Y_{TP} and A_{AR}, (**B**) Y_{TP} and P_R, (**C**) Y_{TFn} and A_{AR} and (**D**) Y_{TFn} and P_R.

4. Conclusions

In this study, it was shown for the first time that a novel bio-based DES, composed of L-lactic acid and nicotinamide, in combination with ultrasonication, can be a highly performing solvent for the

extraction of antioxidant polyphenols from various medicinal plants. DES comprised of L-lactic acid and L-alanine was also very promising in this regard, providing yields significantly higher than those attained with water or aqueous ethanol. Mixtures of all the DES tested with β-CD showed that such media could boost polyphenol extraction, but this depended on the nature of the DES. The picture seen when the yield in total flavonoids was considered, was fundamentally different, indicating that β-CD addition to the DES tested might have variable and unpredictable impact. Correlation of the antiradical activity and reducing power of the extracts generated was higher with the total polyphenols, suggesting that higher extractions yields also entailed more powerful antioxidant effects.

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Author Contributions: C.G. and A.-E.L. carried out the laboratory work; N.P. cultivated and handled the medicinal plants used; D.P.M. performed the experimental design, handled and processed the data, and wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

Nomenclature

A_{AR}	antiradical activity (μmol DPPH g^{-1})
P_R	reducing power (μmol AAE g^{-1})
$R_{L/S}$	liquid-to-solid ratio (mL g^{-1})
$R_{mol}^{D/A}$	molar HBD:HBA ratio (dimensionless)
T	temperature (°C)
Y_{TFn}	yield in total flavonoids (mg RtE g^{-1})
Y_{TP}	yield in total polyphenols (mg CAE g^{-1})

Abbreviations

AAE	ascorbic acid equivalents
DES	deep eutectic solvent
DPPH	2,2-diphenyl-1-picrylhydrazyl radical
CAE	caffeic acid equivalents
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
TPTZ	2,4,6-tripyridyl-*s*-triazine

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