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Effect of Natural Deep Eutectic Solvents on Phenolic Extraction Efficiency and Antioxidant Activity of Date Seeds Extract

Heba A. Sindi [1,*], Alanood A. Alfaleh [1] and Marya J. Hamdan [1]

[1] Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah, Saudi Arabia; hasindi@kau.edu.sa, aaalfaleh@kau.edu.sa, mabedhamdan@stu.kau.edu.sa
* Correspondence: he_sindi@kau.edu.sa

Abstract: A single Natural deep eutectic solvent (NADES) have emerged as eco-friendly alternatives to traditional toxic organic solvents, which offer enhanced extractability and bioavailability of bioactive compounds found in wasted date seeds. The aim of the study was to investigate the effect of NADES efficiency on the antioxidant activity of extracted date seeds; The physiochemical properties of three prepared NADES based on L-proline as a hydrogen bond acceptor (HBA) and different hydrogen bond donors (HBD) of glycerol, lactic acid, and citric acid were measured, then used to extract (1 g) of date seeds powder using ultrasound method, compared with conventional solvents (water and 70% ethanol). The total phenolic content (TPC) and antioxidant activity, using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, of date seeds extracts were evaluated spectrophotometrically; The highest yield of TPC for date seeds extract was found in lactic and citric acids-based NADES (38.31 ± 0.06 and 30.44 ± 0.85 mg GAE/g dw, respectively), with also the highest antioxidant activity (90.96 ± 0.48 and 90.61 ± 0.16%, respectively) compared with other solvents; The results revealed that carboxylic acid-containing NADES enhanced effectiveness, which hold promise for polyphenols extraction from date seeds with distinct antioxidant activities. Further research is warranted to explore the potential bioactivities of NADES-based extraction processes.

Keywords: Date seeds extraction; Natural deep eutectic solvents (NADES); total phenolic content; antioxidant activity; phenolic compounds

1. Introduction

Date palm (Phoenix dactylifera L.) seeds, defined as byproduct of date fruit, account for approximately 6- 15% of the date fruit's weight which is estimated annually at 863,000 tons as food waste [1]. This substantial quantity of discarded date seeds represents both an environmental concern and economic loss, given their rich nutraceutical properties of several bioactive compounds, including dietary fiber, proteins, fatty acids, polyphenols, minerals, and vitamins [1,2]. Also, date seeds have been found to contain seven distinct phenolic acids, a key class of polyphenols, namely caffeic, chlorogenic, p-coumaric, ferulic, gallic, syringic, and vanillic acids. Moreover, rutin, quercetin, and luteolin are among the prominent flavonoids of polyphenols found in date seeds [2]. The presence of these bioactive compounds contributes to their numerous health benefits such as antioxidant, antidiabetic, anti-carcinogenic, and anti-inflammatory characteristics [1].

Consequently, the exploration of effective solutions to utilize an agricultural and industrial waste product of date seeds has garnered increasing attention from researchers [3]. However, the extraction process plays a pivotal role in acquiring these valuable compounds [4]. Traditional extraction methods often employ large amounts of hazardous solvents such as methanol, ethyl acetate, or chloroform, posing environmental risks [5]. To address this issue and align with

green consumerism principles, researchers have turned to innovative alternatives such as Natural Deep Eutectic Solvents (NADES) [6,7].

In the quest for sustainable and eco-friendly alternatives to traditional organic solvents, NADES has emerged as a novel bio-based type of green solvent [5,8]. These solvents are liquid mixtures formed through the hydrogen-bonding interaction of two or more natural components of plants' primary metabolites such as sugars, amines, alcohols, organic acids, and amino acids in specific molar ratios [9]. NADES possess a range of desirable characteristics, including sustainability, renewability, biodegradability, low toxicity, non-flammability, and simple preparation at an affordable cost [10,11]. Moreover, the applications of NADES extend to the field of phytonutrients, where they have shown potential for enhanced extraction and improved solubility of bioactive compounds which facilitates the development of functional foods and nutraceutical products [8].

Despite the eco-friendly and sustainable nature of NADES as "greener" alternatives to conventional solvents, there is limited research on their application in the extraction of date seeds. Additionally, the impact of NADES, which can be virtually produced as an unlimited number of potential mixtures, on the properties of the resulting extract, particularly in terms of antioxidant activity, remains largely unexplored. Therefore, the present study aimed to investigate the effect of three different types of NADES on the antioxidant activity of date seeds extract.

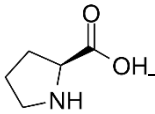
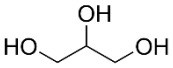
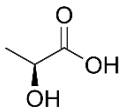
2. Materials and Methods

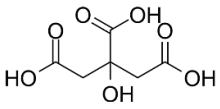
2.1. Materials

Samples of date palm seeds, chemicals, reagents, and equipment used in the study were published previously by Alfaleh and Sindi [12]. L-proline (Pro, ≥ 99.0%) was provided by Solarbio Science & Technology Co., Ltd. (Beijing, China). 2.3. Preparation for NADES

The preparation of NADES was according to Karadendrou et al. [13]. In screw-capped bottles, L-proline as a hydrogen bond acceptor (HBA) with alcohol (glycerol) and carboxylic acids (lactic and citric acids) as hydrogen bond donors (HBD) were mixed at molar ratios of Pro-Gly (1:2), Pro-La (1:2), and Pro-Citri (1:1) in a water bath at 60°C for an hour homogenizing. The obtained mixtures were heated and stirred at 60°C using a hotplate stirrer until clear liquids were formed as revealed in Table 1 and Figure 1. Then, all NADES were diluted with 20 or 30% (v/v) distilled water and kept in sealed containers at 20°C.

Table 1. Natural deep eutectic solvents composition and preparation conditions.

HBA1	HBD1	Acronyms (Molar Ratio)	Condition	Water Added (%) *	Appearance
 L-proline	 Glycerol	Pro-Gly (1:2)	60°C 3 h	20	Transparent orange-yellow viscous liquid
	 Lactic acid	Pro-La (1:2)	60°C 1 h	20	Transparent yellow semi-viscous liquid

 Citric acid	Pro-Citri (1:1)	60°C 12 h	30	Transparent light brown viscous liquid

1HBA: hydrogen bond acceptor; HBD: hydrogen bond donor. * Percentage of water added based on the NADES volume (v) in ml. [14]1

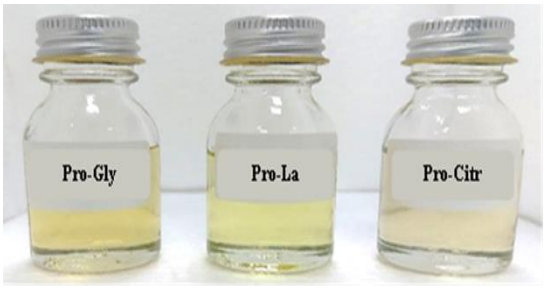


Figure 1. Natural deep eutectic solvents prepared from L-proline (Pro) as hydrogen bond acceptors with glycerol (Gly), lactic acid (La), and citric acid (Citri) as hydrogen bond donors.

2.4. Preparation of Date Seeds Sample

Date seeds powder was prepared as published previously by Alfaleh and Sindi [12]. Date seeds cleaned and washed, then dried in an oven for 12h at 50°C. After that seeds grounded and sifted to size 0.5-mm particles.

2.5. Extraction of Date Seeds Powder

Date seeds powder was extracted with the prepared NADES (Pro-Gly, Pro-La, or Pro-Citri) or conventional solvents (water or 70% ethanol) using the method published previously by Alfaleh and Sindi [12]. Date seeds powder was mixed in a ratio of 1:10 (weight:volume), the mixture was mixed at a speed of 800 rpm for 3 min. The extracts were then transferred to the ultrasound bath at 40°C for 30 min. The extracts were centrifuged at (2100× g) for 15 min and kept at − 20°C until use.

2.6. Physiochemical Properties of NADES

The measurements of pH, viscosity, and polarity of NADES and conventional solvents (5 samples) were carried out in triplicate following the method published previously by Alfaleh and Sindi [12] with minor modification, which involved using a spindle number 1 (S61) for low-viscosity NADES (Pro-La) and a spindle number 2 (S62) for high-viscosity NADES (Pro-Gly and Pro-Citr).

2.7. Fourier-transformed Infrared (FTIR) Analysis

The prepared NADES and their components (7 samples) were evaluated for Fourier-transform Infrared (FTIR) spectroscopy using an FTIR spectrometer (Thermo Fisher Scientific Inc., USA).

The samples were scanned over the infrared light path at 20°C and then FTIR spectra of the wavenumber from 4000 to 500 cm-1 were collected for chemical properties analysis [15].

2.8. Total Phenolic Content (TPC) by Folin-Ciocalteu Method

The total phenolic content (TPC) of five samples in triplicate of date seeds extracted with NADES or conventional solvents was evaluated using the Folin-Ciocalteu reagent method as published previously by Alfaleh and Sindi [12]. The samples were diluted using phosphate buffer (pH 7), and 100 µL of extracts mixed with 200 µL of Folin–Ciocalteu's reagent (10%; v/v). After 5 min, 2000 µL of 7.5% sodium carbonate solution added and vortexed, then incubated for 2 h at room temperature in dark place. Samples measured at wavelength 765 nm using UV–vis spectrophotometer.

2.9. Antioxidant Activity by DPPH Scavenging Assay

The antioxidant activity of five samples of date seeds extracted with NADES or conventional solvents was measured using the DPPH free radical scavenging assay following the procedure published previously by Alfaleh and Sindi [12]. 100 µL of the extract or the control mixed with 1500 µL of the DPPH solution then the mixtures kept for 1 h at room temperature in the dark. Finally, samples diluted using the phosphate buffer (pH 7) and measured at 517 nm.

2.10. HPLC- UV/VIS Analysis

Reversed phase (RP) high-performance liquid chromatography (HPLC) was employed to detect and quantify polyphenols in four different date seeds extracts according to the method published previously by Alfaleh and Sindi [12]. Using system

(Shimadzu, Kyoto, Japan) and C18 column (250 × 4.6 mm × 5 µm), UV–VIS detector. The column was incubated at 40°C. The mobile phase consists of solvent A (acetonitrile) and solvent B (water with acetic acid, 99:1, v/v, pH 2.30). (20 µL) of samples injected at a flow rate of 1 mL/min. Gradient method used as following (20% A (5 min), 80% A (10 min), 20% A (5 min)). The detector was set at 280 nm for gallic acid, catechin, syringic acid, p-coumaric acid, and 370 nm for rutin, quercetin.

2.11. Statistical Analysis

The data were expressed as mean ± standard deviation (SD) of triplicate performed tests. The experimental results were analyzed for statistical comparisons by one-way analysis of variance ANOVA test, and the significance of the difference between means was assessed by Duncan's multiple range test. The analysis was applied using the statistics software SPSS for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Physiochemical Properties of NADES

Table 2 shows three of the main physicochemical characteristics of the studied NADES (Pro-Gly, Pro-La, and Pro-Citri) in comparison with conventional solvents (water and 70% ethanol). The choice to study pH, viscosity, and polarity as important parameters is justified because they have a substantial influence on chemical reactions and applications like solubility and catalysis.

Table 2. Physicochemical characteristics of natural deep eutectic and conventional solvents.

Solvents	Acronyms	Physicochemical Characteristics		
		pH	Viscosity (cP)	ENR (kcal/mol-1)
NADES	Pro-Gly	6.64 ± 0.03 b	246.0 ± 1.0 b	49.49 ± 0.28 b
	Pro-La	2.69 ± 0.01 d	65.67 ± 0.12 c	49.18 ± 0.05 c
	Pro-Citri	2.11 ± 0.03 e	308.0 ± 1.0 a	48.00 ± 0.05 d
Conventional	Water	7.17 ± 0.31 a	0.87 ± 0.07 d	43.17 ± 0.16 e
	70% EthOH	6.13 ± 0.25 c	1.13 ± 0.16 d	50.89 ± 0.19 a

NADES: natural deep eutectic solvents; Pro: L-proline; Gly: glycerol; La: lactic acid; Citri: citric acid; EthOH: ethanol; cP: centipoise; ENR: electron transition in Nile red. Values are the mean of three triplicates ± SD. Different letters in the same column indicate significant differences ($p < 0.05$). Results show significant increase in viscosity of NADES.

3.1.1. pH Value

The investigation of pH values in NADES provides valuable insights into their acidity or alkalinity as presented in Table 2. According to Nugrahani and Jessica [16], L-proline, an amino acid, possesses both acidic and basic functional groups, but its overall pH tends to be close to neutral when combined with other solvents or compounds. The results revealed that Pro-Gly had a pH of 6.64 ± 0.03, which is supported also by Karadendrou et al. [13] who found the pH of alcohol-based NADES indicated a nearly neutral nature. In contrast, Pro-La and Pro-Citri exhibited a significantly lower pH of 2.69 ± 0.01 and 2.11 ± 0.03, respectively, suggesting a highly acidic composition. The strong acidity observed is probably a result of the carboxylic functional groups found in HBD [11,13,17]. Also, the combination of L-proline and carboxylic acid likely enhances the overall acidity of NADES, resulting in the observed low pH value [9].

3.1.2. Viscosity

In Table 2, the viscosity of NADES at 30 rpm revealed significant variations of 65.67, 246.0, and 308.0 cP for Pro-Citri exhibited the highest value, followed by Pro-Gly and Pro-La. The nature of the HBD component affects the viscosity of NADES, which has been confirmed by various studies [13,15]. According to Fuad et al. [9], the viscosity of NADES is higher with carboxylic acid compared to alcohol due to the solid state of acids (excluding lactic acid), while glycerol is utilized in NADES as a liquid state. On the other hand, Pro-Citri has a higher viscosity than Pro-La attributed to the larger molecular size and three carboxylic groups of citric acid [9] that have stronger interactions and more hydrogen bonds with L-proline. In contrast, lactic acid is a smaller molecule with a single carboxylic group [11], resulting in weaker intermolecular interactions and lower viscosity.

Moreover, it was observed that all NADES exhibited significantly higher viscosity compared to the conventional solvents ($p < 0.05$), caused by the extensive hydrogen bonding which promotes stable molecular interactions between their components [9,17]. However, the high viscosity of NADES hinders the mass transfer and diffusion of the target solutes into the solvent, resulting in poor extraction efficiency [18]. To mitigate this, adding a small amount of water (less than 50%) weakens hydrogen bonds, effectively reducing viscosity without sacrificing the eutectic properties

of NADES [10,19]. Additionally, selecting an appropriate molar ratio of the HBA and HBD components can also be crucial to avoid excessively high viscosity or saturation [11].

3.1.3. Polarity

The results from Table 2 indicate the polarity of NADES by ENR values where a high value represents a low polarity. Carboxylic acid-containing NADES (Pro-Citri and Pro-La) exhibited the highest polarity, with ENR values of 48.00 and 49.18 kcal/mol-1, respectively, while alcohol-based NADES (Pro-Gly) showed the lowest polarity of 49.49 kcal/mol-1. These findings align with previous studies that have reported the polar nature of solvents is closely linked to the occurrence of hydrogen bonds [6,9,13]. Consequently, an increase in the number of hydrogen bonds is expected to enhance the polarity of NADES in which carboxylic groups demonstrated a superior hydrogen bonding ability.

Furthermore, all three NADES demonstrated a higher polarity than 70% ethanol (ENR < 50.89, $p < 0.05$) but a lower polarity than water (ENR > 43.17, $p < 0.05$), attributed also to the variations in the molecular structures and interactions [13]. Also, the addition of water to NADES has an impact on their polarity, leading to a decrease in ENR values, which can be explained by the disruption of the molecular structure of NADES caused by the rupture of hydrogen bond interactions between the HBA and HBD components [9,19].

3.2. FTIR Spectra of NADES

The FT-IR spectra of the mixture of L-proline and glycerol (Pro-Gly) and their individual IR spectra are depicted in Figure 2. By combining these results, it was found that there is an H-bond interaction between the functional groups of glycerol and L-proline appeared in the increase of broadness for the hydroxyl group (-OH) region from 3500 to 2600 cm-1 in Pro-Gly. Also, the upward shift of an absorption band of the amine functional group (N-H) was noted from 1610 to 1615 cm-1 [19,20].

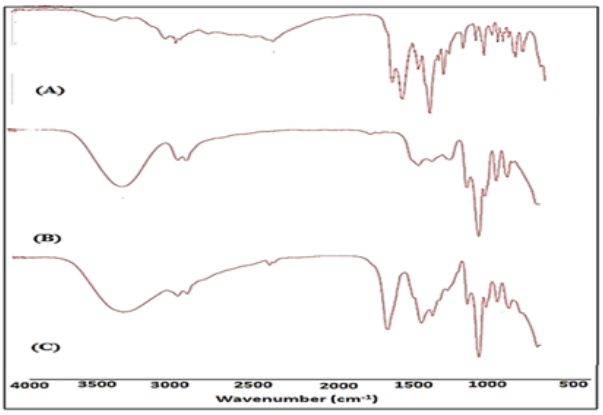


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Figure 2. Fourier-transformed infrared spectra of natural deep eutectic solvent and its components (A) L-proline (Pro); (B) Glycerol (Gly); (C) Pro-Gly

Similarly, the IR data depicted in Figure 3 for L-proline, lactic acid, and their mixture (Pro-La) showed a very broad band at 3500-2500 cm-1 in Pro-La, and the appearance of an absorption band at 1605 cm-1 in the mixture Pro-La indicated the formation of intermolecular H-bond between L-proline and lactic acid [20].

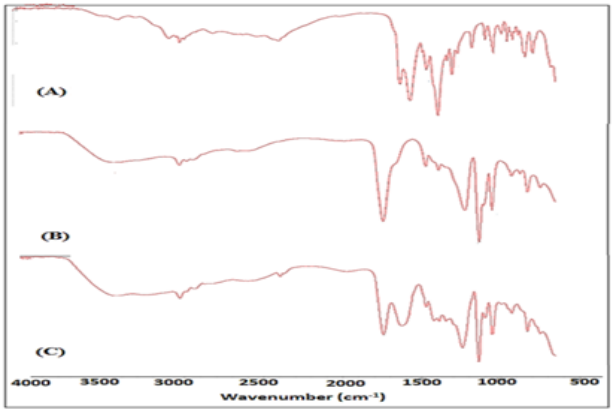

Figure 3. Fourier-transformed infrared spectra of natural deep eutectic solvent and its components (A) L-proline (Pro); (B) Lactic acid (La); (C) Pro-La. The results indicated the formation of intermolecular H-bond between L-proline and lactic acid.

Finally, the last NADES consists of L-proline and citric acid (Pro-Citri) shown in the IR spectrum in Figure 4. By examining the IR charts of the solvent Pro-Citri and its components, it was found that there is a broad absorption band for the hydrogen-bonded hydroxyl functional groups (-OH) in Pro-Citri at 3600-2400 cm-1 which differs from their procurers L-proline and citric acid. In addition, there was a downward shift of the carbonyl functional group (C=O) absorption band from 1742 and 1696 cm-1 (for citric acid) to 1721 cm-1 in the mixture Pro-Citri. The amine functional group (N-H) absorption band appeared also in the mixture with a shift from 1610 to 1606 cm-1 [19].

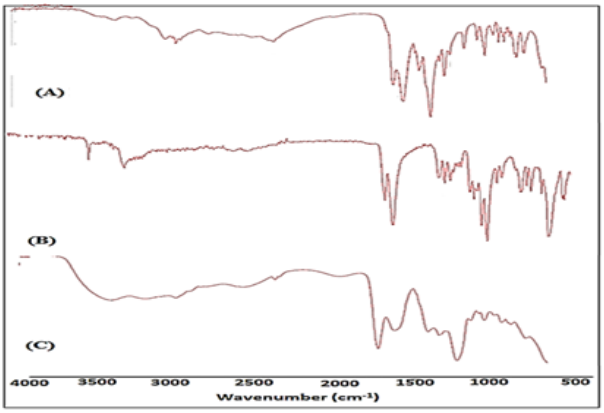

Figure 4. Fourier-transformed infrared spectra of natural deep eutectic solvent and its components (A) L-proline (Pro); (B) Citric acid (Citri); (C) Pro-Citri. The results show downward shift of the carbonyl functional group (C=O) absorption band (for citric acid) compared to the mixture Pro-Citri, also amine functional group (N-H) absorption band appeared also in the mixture with a shift

3.3. Spectrophotometric Determination of TPC in Date Seeds Extract

The study employed L-proline-based NADES consisting of either carboxylic acids or alcohol as HBD, as shown in Table 1. These NADES exhibited a viscous or semi-viscous transparent appearance. To enhance the extraction performance of NADES, the optimal extraction media included a concentration of 20 or 30% distilled water. This choice aimed to reduce the viscosity of NADES, enhance the mass transfer rate, improve the extraction yield, and facilitate better handling of the solvents [18,19].

Additionally, this study considered the desirable extraction temperature range, which typically falls between 25 °C and 60 °C [20]. In this investigation, ultrasound-assisted extraction was employed for 30 min at a temperature of 40 °C to achieve optimal yield efficiency of phenolic compounds from the date seeds powder [15,22].

The use of ultrasound enhances the extraction yield of bioactive compounds by utilizing acoustic cavitation, which generates bubbles, increases pressure and temperature, and disrupts the cell wall matrix of the date seeds. This disruption promotes solvent penetration and allows for the release of bioactive compounds [23].

Furthermore, the extraction ability of NADES for bioactive compounds is influenced by various physicochemical characteristics, including pH, polarity, and the nature of interactions between HBA and HBD [24]. The results found that NADES containing lactic acid (Pro-La) exhibited the highest extraction efficiency on date seeds, followed by citric acid-based and glycerol-based NADES (Pro-Citri and Pro-Gly). The yields of TPC for the three NADES were 38.31 ± 0.06, 30.44 ± 0.85, and 29.37 ± 3.74 mg GAE/g dw, respectively (Table 3). The conventionally used solvents (water and 70% ethanol) resulted in lower amounts of TPC compared to NADES ($p < 0.05$). Specifically, the TPC values obtained were 20.58 ± 0.47 mg GAE/g dw for water and 22.94 ± 0.76 mg GAE/g dw for 70% ethanol.

Table 3. Total phenolic content and antioxidant activity of date seeds extracted with natural deep eutectic and conventional solvents.

Date Seeds Extracts	Acronyms	TPC	DPPH
		(mg GAE/g dw)	(%)
NADES extraction	Pro-Gly + DS	29.37 ± 3.74 b	44.36 ± 0.39 b
	Pro-La + DS	38.31 ± 0.06 a	90.96 ± 0.48 a
	Pro-Citri + DS	30.44 ± 0.85 b	90.61 ± 0.16 a
Conventional extraction	Water + DS	20.58 ± 0.47 c	45.95 ± 1.40 b
	70% EthOH + DS	22.94 ± 0.76 c	45.74 ± 1.38 b

NADES: natural deep eutectic solvents; Pro: L-proline; Gly: glycerol; La: lactic acid; Citri: citric acid; EthOH: ethanol; DS: date seeds; TPC: total phenolic content; GAE: gallic acid equivalents; dw: dry weight; DPPH: 1,1-diphenyl-2-picrylhydrazyl. Values are the mean of three triplicates ± SD. Different letters in the same column indicate significant differences ($p < 0.05$). The results show significant increase in TPC and DPPH for date seeds extracted with NADES solvents compared with date seeds extracted with conventional solvents.

The superior extractability of NADES can be attributed to the presence of multiple hydrogen bonding interactions that enhance the solvation and dissolution of the phenolic compounds in NADES, leading to higher extraction yields [6,25]. However, NADES with high viscosity exhibited lower extractability efficiency of phenolics due to hindered diffusion and mass transfer caused by the increased intermolecular forces [22,26]. The lower viscosity observed previously in lactic acid-containing NADES (Table 2), which has one carboxyl group, compared to NADES formed by citric acid (three carboxyl groups) and glycerol (multiple hydroxyl groups) may explain the higher extraction efficiency of lactic acid-based NADES in extracting phenolic compounds on date seeds.

Furthermore, the pH and polarity of NADES also played a role, with more acidic and polar NADES leading to higher extraction yields [17,27]. In agreement with Airouyuwa et al. [15], carboxylic acid-based NADES were found to be more polar than alcohol-based NADES (Table 2), which resulted in the optimal yields of TPC. Overall, these findings suggest that the choice of solvent significantly impacts the extraction efficiency in which all tested NADES demonstrated higher extractability performance of polyphenols on date seeds compared to the conventional solvents.

3.4. Antioxidant Activity of Date Seeds Extract

The current study evaluated the antioxidant activity of date seeds extracted with three different NADES using the DPPH free radical scavenging assay (%). It is worth noting that among the various HBD used in L-proline-based NADES, the carboxylic acids (Pro-La and Pro-Citri) exhibited higher antioxidant activity of 90.96 ± 0.48% and 90.61 ± 0.16%, respectively, compared to alcohol (Pro-Gly) with antioxidant activity of 44.36 ± 0.39% ($p < 0.05$), as shown in Table 3.

The findings are consistent with Airouyuwa et al. [15], which reported significantly higher antioxidant activity in extracts from date seeds (1:30 g/ml) using carboxylic acid-based NADES at the same ratio as in this study (1:2). He et al. [28] also obtained good results with Pro-La (1:1) for Salvia miltiorrhiza herbal plant, achieving a DPPH scavenging effect of about 87%, while Mansinhos et al. [22] achieved 56.96% for Lavandula plant. These high antioxidant activities can be attributed to the abundant phenolic compound content, which possesses radical scavenging properties in natural products [29]. Several studies have attributed the high antioxidant activity of NADES extracts to their ability to effectively recover bioactive compounds [11,25].

Furthermore, the extraction efficiency of NADES was compared to that of conventional solvents of water and 70% ethanol, using the same extraction techniques. Previous studies have suggested that NADES can serve as efficient alternatives to conventional solvents [9,15,27]. NADES can form hydrogen bonds with the target molecules during extraction, facilitating electron donation and acceptance, thereby enhancing the mass transfer rate [6,27]. In Table 3, NADES based on lactic and citric acids exhibited significantly higher extraction efficiency compared to the conventional solvents ($p < 0.05$). However, no significant difference was observed for glycerol-based NADES.

Generally, the antioxidant capacity of NADES extracts increases with the amounts of extracted compounds. Nevertheless, factors such as the type of NADES and its molar ratio indirectly influence the antioxidant capacity [9]. According to that, the lower antioxidant activity observed for Pro-Gly NADES, despite having a higher yield of TPC compared to the conventional solvents, could be attributed to the lack of synergistic effects between L-proline and glycerol, resulting in suboptimal solubility of certain polyphenols in NADES and then lower antioxidant activity due to limited interactions with the solvent.

Moreover, it could be for the possibility of extracting different classes of polyphenols with varying antioxidant activities compared to other solvents. Overall, it is crucial to consider the composition, solubility, and specific antioxidant mechanisms of the extracted polyphenols when evaluating their antioxidant activity. Further studies and characterization are needed to better

understand the reasons behind these differences and optimize the extraction process for improved antioxidant activity.

3.5. Identifies and Quantifies Phenolic Compounds of Date Seeds Extract by HPLC

Table 4 shows the concentration by mg/100 g dw of date seeds for six polyphenols (phenolic acids and flavonoids) in date seeds extracted by two NADES (Pro-La and Pro-Citri) and two conventional solvents (water and 70% ethanol).

Table 4. Polyphenols compounds concentration (mg/100 g dry weight of date seeds) in date seeds extracts using natural deep eutectic solvents by HPLC.

Date Seeds Extracts	Polyphenolic Compounds Concentration (mg/100 g dw)					
	Gallic acid	**Catechin**	**Syringic acid**	***p*-coumaric**	**Rutin**	**Quercetin**
Pro-La + DS	n.d	236.84 ± 10.45 ^{a}	n.d	n.d	n.d	5.73 ± 0.41 ^{b}
Pro-Citri + DS	509.71 ± 5.48 ^{b}	n.d	113.68 ± 0.86 ^{b}	89.23 ± 0.07 ^{b}	5.31 ± 0.36 ^{b}	8.69 ± 0.55 ^{a}
Water + DS	860.99 ± 0.01 ^{a}	n.d	n.d	n.d	n.d	9.43 ± 0.04 ^{a}
70% EthOH + DS	349.56 ± 15.79 ^{c}	n.d	128.38 ± 2.10 ^{a}	128.18 ± 0.73 ^{a}	86.01 ± 4.37 ^{a}	4.86 ± 0.00 ^{b,c}

Pro: L-proline; La: lactic acid; Citri: citric acid; EthOH: ethanol; DS: date seeds; dw: dry weight; n.d: not detected. Values are the mean of 2 duplicates ± SD. Different letters in the same column indicate significant differences (Duncan's multiple-range test, $p < 0.05$).

The results indicate that date seeds extracted with water and Pro-Citri NADES exhibit significantly greater concentrations of gallic acid at 860.99 ± 0.01 and 509.71 ± 5.48 mg/100 g dw, respectively, compared to other extracts of date seeds. The concentration of catechin was indicated in the Pro-La extraction of date seeds at 236.84 ± 10.45 mg/100 g dw, while no concentrations of catechin were found in the conventional solvents and Pro-Citri extraction of date seeds. The concentration of syringic acid and *p*-coumaric acid were found in date seeds extracted with 70% ethanol and Pro-Citri NADES for both compounds. The concentration of syringic acid in 70% ethanol extract was 128.38 ± 2.10 mg/100 g dw, and in Pro-Citri extract was 113.68 ± 0.86 mg/100 g dw, while the concentration of *p*-coumaric acid in 70% ethanol extract was 128.18 ± 0.73 mg/100 g dw, and in Pro-Citri extract was 89.23 ± 0.07 mg/100g dw.

The findings also showed that there was no statistically significant difference ($p = 0.269$) in the levels of quercetin between water extraction and Pro-Citri extraction of date seeds, with concentrations of 9.43 ± 0.04 and 8.69 ± 0.55 mg/100g dw, respectively. However, both extracts had higher concentrations compared to other extracts. The rutin had higher concentrations in 70% ethanol extract (86.01 ± 4.37 mg/100g dw) than the values in other extracts of date seeds. The results showed that the concentrations of phenolic compounds in the current study were exhibited in date seeds extracted with 70% ethanol and Pro-Citri NADES both recognized five phenolic compounds out of six.

Date seeds are rich in various polyphenolic compounds, such as rutin, quercetin, catechin, 4-Hydroxybenzoic acid, gallic acid, caffeic acid, syringic acid, p-coumaric acid, and other chemicals being the most notable among them according to HPLC analysis [30,31,32], the amounts and types of the polyphenols depend on various factors, including the source and species of the crop [33], as well as the techniques and solvents employed for extraction [34], this elucidates the underlying cause for the disparities in HPLC findings across various studies.

The most notable result in the present results was for date seeds extracts obtained using Pro-Citri NADES. The finding aligns with a study conducted to estimate seventeen polyphenolic compounds in the *Buddleja Globosa* hope leaves using eight different NADES. The results demonstrated that NADES containing L-proline and citric acid exhibited higher concentrations of polyphenolic compounds compared to other solvents examined [35]. Moreover, Duan et al. [36] documented that NADES containing L-proline can extract phenolic compounds derived from five various Chinese herbal remedies more effectively than ethanol. Ali et al. [37] and Maimulyanti et al. [38] suggested that L-proline-based NADES can form hydrogen bonds to extract.

polar compounds. Lu et al. [39] found that NADES containing carboxyl acids as hydrogen donors contain free H+ ions which enhance solvation power and interact with polar compounds in date seeds extracts. These ions can promote the hydrolysis of cellulose, hemicellulose, and pectin in cell walls, leading to a decrease in the density of natural resource tissues. As a result, phenolic compounds can readily diffuse from the material matrix into the solvent.

4. Conclusions

The study presented the enhanced effectiveness of NADES over conventional solvents (water and 70% ethanol) in terms of enhanced extractability of polyphenols and antioxidant activity in date seeds. NADES consisting of L-proline with carboxylic acids (Pro-La and Pro-Citri) showed the highest extraction yields of TPC from date seeds, which revealed the highest antioxidant activity compared to other solvents. Furthermore, a study on phenolic compounds in date seeds extracts revealed that the primary phenolic compounds identified were gallic acid and catechin. Additionally, minor levels of syringic acid, rutin, p-coumaric acid, and quercetin were also present in the date seeds extracts. The concentrations of these phenolic compounds were found to be highest in the date seeds extracts obtained using 70% ethanol and Pro-Citri NADES. In terms of identification, both the 70% ethanol and Pro-Citri extraction of date seeds successfully identified five out of the six phenolic compounds studied.

Replacing traditional hazardous solvents with NADES will contribute to the development of sustainable extraction methods and align with the green consumerism principles for utilizing date seeds as a rich source of polyphenols with various health benefits. Extracting these compounds using NADES not only helps in valorizing date seeds and reducing food waste but also develops the potential of functional food products and nutraceutical formulations. In addition, extracting phenolic compounds using NADES can be used in future to develop food or drink products such as energy bars and tea, juice, energy or dairy drinks. It could be added as natural supplements to improve health or to improve digestion by enhancing gut microbiota balance.

The study is limited, using only one type of date seeds, more varieties of date seeds sources to assess the antioxidant and antibacterial potential of date seeds extracts.

The study examined a limited number of NADES formulations, further investigation is needed to determine the efficiency and bioactivity of other NADES solvents formulation. The study primarily focused on polyphenols, further investigation is needed on other potentially beneficial bioactive compounds present in date seeds extracts, warranting a more holistic approach to compound identification.

For the study recommendations, further analysis is needed to examine the NADES effect on the antimicrobial activity of date seeds extracts for assessing their potential as alternative

antimicrobial agents. Finally, future research can explore a wider range of NADES formulations to optimize the extraction process and evaluate their feasibility in industrial settings for the betterment of human health and well-being.

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تأثير المذيبات الطبيعية على كفاءة استخلاص المركبات الفينولية والنشاط المضاد للأكسدة لمستخلص نواة التمر المؤلفين

هبة عباس سندي[١]، العنود عبدالعزيز الفالح[١]، مارية جمال حمدان[١]

[١]قسم الغذاء والتغذية، كلية علوم الإنسان والتصاميم، جامعة الملك عبدالعزيز، جدة– المملكة العربية السعودية

بيانات الباحث المراسل

هبة عباس سندي

he_sindi@kau.edu.sa

الملخص:

ظهرت المذيبات الطبيعية (NADES) كبدائل صديقة للبيئة للمذيبات العضوية التقليدية السامة، حيث تعزز عملية الاستخلاص والتوافر الحيوي للمركبات الفينولية الموجودة في بذور التمر المهدرة، وقد هدفت هذه الدراسة إلى بحث تأثير كفاءة المذيبات الطبيعية على استخلاص المركبات الفينولية من نواة التمر والنشاط المضاد للأكسدة لها. تم قياس الخصائص الفيزيائية والكيميائية لثلاث أنواع محضرة من المذيبات الطبيعية المستندة إلى البرولين كمستقبل للرابطة الهيدروجينية مع حمض اللاكتيك أو حمض الستريك أو الجليسرول كمانحات للرابطة الهيدروجينية، لاستخلاص (١جم) من مسحوق بذور التمر باستخدام الموجات فوق الصوتية مقارنةً بالمذيبات العضوية التقليدية (الماء، الإيثانول ٧٠%). تم تقدير إجمالي تركيز المركبات الفينولية (TPC) والنشاط المضاد للأكسدة لمستخلصات بذور التمر باستخدام اختبار ١,١–ثنائي فينيل–٢–بيكرايل هيدرازيل (DPPH) عن طريق قياس الطيف الضوئي. أظهرت النتائج أن أعلى تركيز للمركبات الفينولية وُجِد في بذور التمر المستخلصة باستخدام المذيبات الطبيعية المستندة على حمض اللاكتيك وحمض الستريك (٣٨,٣١ ± ٠,٠٦ و ٣٠,٤٤ ± ٠,٨٥ ملجم لمكافئ حمض الغاليك/ جم من الوزن الجاف، على التوالي)، والتي كشفت أيضًا عن أعلى نشاط مضاد للأكسدة (٩٠,٩٦ ± ٠,٤٨ و ٩٠,٦١ ± ٠,١٦%، على التوالي) مقارنة بالمذيبات الأخرى. أظهرت النتائج التأثير المتفوق للمذيبات الطبيعية التي تحتوي على الأحماض الكربوكسيلية على تحسين كفاءة الاستخلاص، ويمكن التنبوء باستخدام واعد في استخلاص المركبات الفينولية من نواة التمر بنشاط مضاد للأكسدة متميز، مع ضرورة إجراء المزيد من البحوث لاستكشاف الأنشطة الحيوية المحتملة لعمليات الاستخلاص القائمة على مذيبات الطبيعية

الكلمات المفتاحية:

المذيبات الطبيعية، مستخلصات بذور التمر، تركيز المركبات الفينولية، النشاط المضاد للأكسدة، المركبات الفينولية.