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# Application of Choline Chloride as Natural Deep Eutectic Solvents for the Green Extraction of Phenolic Compounds from Rheum Ribes Leaves

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## ABSTRACT

Deep eutectic solvents are applied as a new type of sustainable solvents.The new generation of green solvents are considerable utilize to extraction of bioactive compounds due to their physicochemical properties and structure. In this study, the extraction process of phenolic compounds from rhubarb roots using of ultrasound-assisted extraction (UAE) method was investigated.Totally 8 different types of choline chloride- and L-proline-based deep eutectic solvents containing various H-bond donors/acceptors were synthesized.The extraction conditions for phenolic acids such as effect of different DESs,extraction temperature,water content in nades,ratio of solid to liquid and extraction time were studied.The optimal parameters were found to be extraction time of 30 min, temperature of 313.15 K, DES 30 wt % water and solid/liquid ratio of 1:20 led to significantly effect on the extraction of phenolic contents from plant material. The quantification and characterization of two biactive flavonoids in the extracts was analyzed via ultra-high pressure liquid chromatography (UPLC) with photodiode dedection.Compared with conventional extraction system, deep eutectic solvents (DESs) showed high extraction efficiency for both polar and less polar compounds.

This study reveals,the developed DESs have significant as alternative new green dissolving agents in the extraction of phenolic compounds from plant resource due to their high solubilization ability and low toxicity.

## 1. INTRODUCTION

Environmentally friendly (or green) solvents have a new type of fluids known as deep eutectic solvents (DES).DESs were discovered in 2003 by abbott et.al. [1]. DESs and DES-based material are alternatives to traditional solvents which are often toxic, environmentally unfriendly,volatile poorly biodegraded and flammable [2].The new generation of liquids show many attractive physicochemical properties: low melting point, high solubility, biodegradable, nonvolatility, thermal stability and easy synthesized which can be composed of natural substances (e.g., salts, amino acids, organic acids and sugar, etc.) [3-6]. These solvents are consist of two or more salts that are melting point of eutectic mixtures salts are much lower than its starting substances. However, green deep eutectic solvents have been widely utilized for isolation and extraction of a variety of active compounds from medical plants [7]. The extraction method is also play important role. Ultrasonic assisted extraction method (UAE) has many advantages such as lower cost, higher yield and shorter extraction time, compared to the current extraction techniques [8]. There is capable strong electrostatic interaction between DES and phenolic compounds.Thus, eutectic solvents is show to great potential to extract phenolic substances. We mainly studied on the DES combination of natural compounds such as alcohols, sugar, amine, organic acid and choline chloride. The main feature of choline chloride is that unique component properties such as high solubility, non-toxic, biodegradable quaternary amomnium salt [9]. In a previous study the capability of various ChCl, lactic acid and oxalic acid based DES assisted by microwave were evaluated for extraction of different phenolic compounds from Lonicerae japonicae Flos such as chlorogenic acid, caffeic acid, 3,4-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid [10]. In another similar research, the combination of DES and methanol-water was used to extract the phenolic acids from Herba artemisiae scopariae amoung different 12 DES, tetramethylammonium chloride/urea (1:4 mole ratio) was the best mixture for extraction Park and co-workers [11].

Moreover, phenolic acids was extracted using betaine-, L-proline-, or ChCl-based DES. Totally, for the extraction of different bioactive compounds including phenolic acids from five Chinese herbal medicines different DES combinations were tested [12]. Quercetin, kaempferol, and isorhamnetin also were extracted from Flos sophorae using different ratios

 

of HBA (ChCl, L-proline and citric acid) and HBD (glycerol, xylitol, glucose, adonitol, or malic acid) [13].

Rhubarb (*Rheum ribes L.*) is a plant native to found mostly in eastern Turkey,Lubnanon,India and Iran. Rheum species are medicinal value herbs due to its source of one of anthroquinone.In Turkey, *R.ribesis* is locally called ''ısgın,usgun''.Its roots and leaves have been commonly consumed as raw or cooked. *R.ribesis* roots are used as pharmacetical raw material in the middle east [14,15].

Roots (and leaves) of this plant species are used against variety of diseases such as diabetes [16], obesity [16], hypertension [16], diarrhoea [17] and psychological disorder The roots of this species has been mainly comprised of antioxidant molecules such as quercetin, 5-desoxyquercetin, quercetin 3-O-galactoside, kaempferol-3-O-rhamnoside and quercetin 3-O-rutinoside [18].

In this study,ultrasonic assisted extraction method was utilized and other extraction-conditions including temperature,time and water content in DES were examined.This green method for extraction of two bioactive components in the R.ribes were used. The determination of flavonoid contents (quercetin and kaempferol) in this plant species was carried out utilizing high performace liquid chromatography-photodiode detection.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals and Materials

The R.Ribes flowers were from Elazig, in eastern Turkey.The samples were dried and stored in the dark to use. Citric acid monohydrate, choline-chloride (99.0%), D(+)-glucose (98.0%), D-(+)-sucrose (99.0% ), urea (98.0%), L-(+)-lactic acid( 98.0%) and L-proline (98.0%) were supplied by Sigma-Aldrich. All others materials were used analytical grade.

### 2.2.Instrumentation

The quantify and identify the individual phenolic compounds were determined using HPLC analysis. The separation was carried out on Agilent (1100) UPLC chromatography equipped with a photodiode ,an automatic column temperature oven,an autosampler, a quaternary pump and a Phenomenex C18 column (5 µm, 4.6 x 150 mm,).The mobile phase was water with 0.05% trifluoroacetic acid (A) and acetonitrile (B). The elution performed was set as follow: 0–10 minute, 10% B; 10–20 minute, 30% B; 20–35 minute, 40% B; 35–50 minute, 10% B. The UV spectrum of kaempferol and quercetin were monitored at $\lambda$ = 280 nm. The constant flow rate was 1.0 mL/min.The standarts and samples were filtered utilizing Millipore 0.20 µm filter.The calibration curves were depicted using different concentrations (10-50 ppm) of all compounds by dissolving them in solvent.

### 2.3. Ultrasound-assisted extraction (UAE)

UAE was performed using an ultrasonic equipment (Hielscher Ultrasonics, Teltow, Germany). The DES-based ultrasonic-assisted extraction process was performed as follows: Temperature was ranged from 30 to 65 °C, water content used in DES was between 5 to 50 %, liquid-solid ratio was ranged from 5 to 40 $mLg^{-1}$, extraction was performed 30-65 minutes and ultrasonic power was applied as 100 W and 30 kHz.

### 2.4.Synthesis of deep eutectic solvents

In the present study, 8 kinds of eutectic mixtures were prepared according to known studies [3,19]. Eight different DESs included pure choline chloride-glycerol (ChGly),pure choline chloride- lactic acid (ChLac), pure choline chloride-D(+) glucose (ChGlu), pure choline chloride-urea (ChUr),pure choline chloride-citric acid (ChCA), pure choline chloride-D(+) glucose-citric acid (ChGluCA), pure choline chloride-urea-glycerol (ChUrGly), L-proline-lactic acid (ProLac).

Component mixture were prepared in deionized water and stirred at 60 °C till a transparent liquid obtained.After this time, natural deep eutectic solvents (NADESs) components were shaken again by a vortex for 1 min, and then they were kept in a desiccator [20]. Abbreviations , appearances and molar ratios of the NADESs were displayed in Table 1. Ratios selected as 1:1 as described by Sylwia and Jakup, 2018 [7].

**TABLE 1**
LIST OF TESTED DESs FOR EXTRACTION

| Type | Name | Combination | Molar ratio |
|---|---|---|---|
| DES-1 | ChLac | Choline chloride:lactic acid | 1:1 |
| DES-2 | ChGly | Choline chloride:glycerol | 1:1 |
| DES-3 | ChGlu | Choline chloride:glucose | 1:1 |
| DES-4 | ChUr | Choline chloride:urea | 1:1 |
| DES-5 | ChCA | Choline chloride:citric acid | 1:1 |
| DES-6 | ChCAglu | Choline chloride:citric acid:glucose | 1:1:1 |
| DES-7 | ChUrGly | Choline chloride:urea:glycerol | 1:1:1 |
| DES-8 | ProLac | L-proline:lactic acid | 1:1 |

## 3. RESULTS AND DISCUSSION

### 3.1. Extraction effect of different DESs

In this research, we use L-Cholineproline and choline chloride as the various types of H-bond donors and HBAs to prepare green solvents.Then, the extraction efficiency of eight analytes perform were compared.Based on comparison among all NADESs, ChCI-Lac based DES provided highest solubility (510 mg/mL).

The yields of polar and non-polar analytes continued as follows ChLac > ChGly > ChUrGly≈MeOH > ChUr> ProLac> ChCAGlu>ChGlu> ChCA.

ChLac (359.8 ± 0.02 mg/mL) and ChGly (340.1 ± 0.01 mg/ mL) showed the best extraction efficiency quercetin due to good ability of quercetin to form hydrogen bonds with DES. The solubility quercetin in methanol was 362.9 ± 0.01 mg/mL, 196.7 ± 0.01 mg/mL in ethanol. ProLac, ChCAGlu and ChCA showed lower capacity to dissolve target compounds.

These results are accordance with similar to previous reports [21,22].The special structure of DES is critical for extracting analytes and is based on physicochemical interactions, other factors such as intrinsic properties and stability of the analytes should also be considered. However, there is a large number of hydrogen bonds allowing to be extraction efficiency increased which cause a positive effect the interactions between active compounds and NADES [20].Figure 1 show that the extraction effect of different DESs solvents.

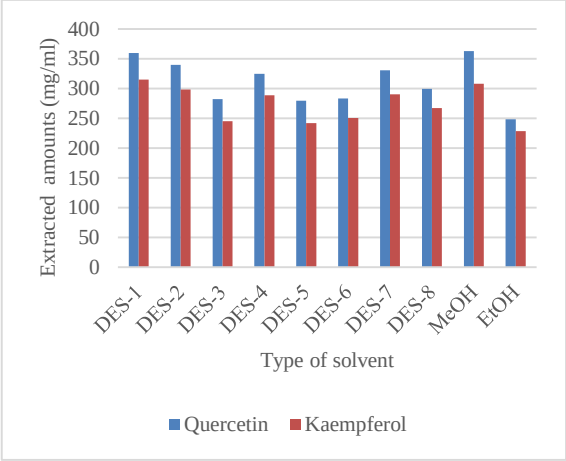


**Figure 1.**The extraction effect of different DESs solvents

### 3.2.Effect of extraction time

Time is an important operational parameter in the completeness of the extraction yield. The sonication time has close related with extraction performed.The effect of extraction time between 15 and 60 min on the target compounds was examined. Figure 2 shows that the bioactive components completely extracted before up to 30 min. and thus, this extraction time was optimum.When the sonication time was increased with above 30 min, extraction performed was decreased.A long extraction time potentially causes solvent polarity to change and also flavonoid contents interaction with ChCI-based DES to form polymer chain structure [23,24].

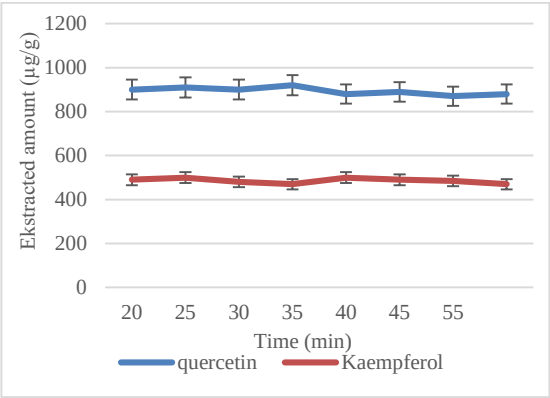


**Figure 2.**The extraction effect of sonication time

### 3.3. Effect of the extraction temperature

Temperature also affects extraction.The extraction temperature was examined between 30°C and 65 °C [25].When the working temperature was higher than 40 °C,the yields were decrease in the amount of all extracted phenol.This is likely because the enhance the solvent viscosity, decrease the mass transfer, which affect the stabilization of the extraction capacity [26].

Although the ultrasonic power performed was achieved. when temperature was increased from 56 to 65 °C.The targeted compounds significantly decomposed,as shown in Figure 3.These results showed a close relationship between extraction efficiency and extraction temperature.

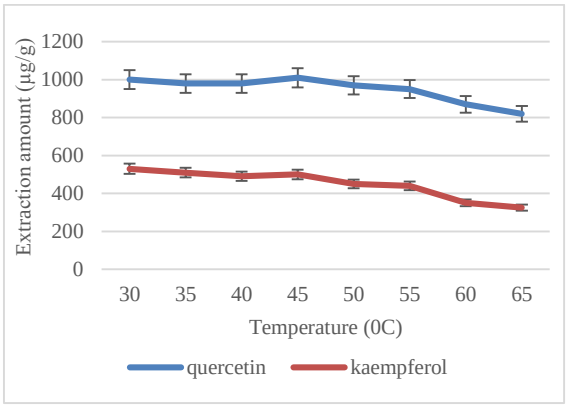


**Figure 3.**The extraction effect of temperature

### 3.4. Effect of water content

The effect of water amount in NADES on the extractability of target compunds was also examined. The addition of water in extraction system can impact on the extraction efficiency [16].The addition of different percentage water could eminently decrease the viscosity and could provide to a positive effect on polar components. With dilutions ranging between %5 and 50% of water content in NADES the extractability of target bioactive flavonoid was examined. As shown in figure 4, the extraction efficiencies of phenolic compounds reduced when the water content to more than 30% increased which cause a negatively impacts interactions between DES and analytes. The excessive high water content would result in the loss of occurring, H-bonds and as a result, interaction between the molecules gradually disappear. Results showed that, a concentration of 30% (v/v) water in NADES could contributed to perform better effects extraction system.

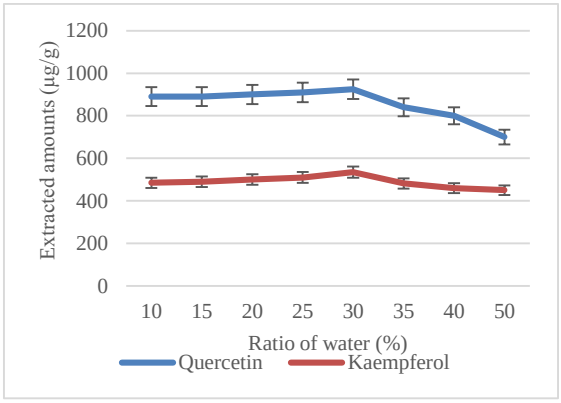


**Figure 4.**The extraction effect of water content

## 4. CONCLUSION

In the experiment,deep eutectic solvents (DESs) was utilized as more effective solvents for the dissolution of compounds,which could be associated with their hydrogen bonding interactions between the medium and components.

The biorenewable eutectic mixtures was established for extraction of quercetin and kaempferol from Rhubarb (*Rheum ribes L.*) roots in high yields.Overall, The parameters of different extraction conditions (i.e solid/liquid ratio,solvent concentration, temperature and time) were provide higher performance extraction for phenolic compounds.

The qualitative identification of individual active components were confirmed by high pressure liquid

chromatography-photodiode. UPLC results showed that better compound separation was observed compared to HPLC methods. Quercetin and kaempferol were the most abundant phenolic acids identified in extract.

The deep eutectic solvents as new green solvents showed a high extraction capacity and efficiently extract bioactive compounds of diverse polarity.

## 5. ACKNOWLEDGEMENT

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## BIOGRAPHIES

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