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Original scientific paper

ULTRASOUND-ASSISTED EXTRACTION OF *ROSA CANINA* L. USING NATURAL DEEP EUTECTIC SOLVENTS

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Abstract

Rosa canina L. (Rosaceae) is frequently employed in traditional medicine, due to its diuretic, anti-inflammatory, anti-allergic, antioxidant, and analgesic properties. The ultrasound-assisted extraction is frequently used as an extraction technique, due to the increased extraction yield, improved extract quality, fast kinetics, and simple operation. Additionally, in recent times, natural deep eutectic solvents (NADESs) are applied as a tool for improving polyphenol recovery from various plant materials. Thus, in the present study, R. canina extracts were prepared using dried rose hips (0.25 g), two types of natural deep eutectic solvents (25 mL, choline chloride+citric acid with 50% of water and betaine+citric acid with 70% of water), and ultrasound probe (40% amplitude for 10 min). The extracts were characterized via analyzing total polyphenol content (TPC), antioxidant potential (ABTS and DPPH assays), extraction yield, pH, zeta potential, conductivity, density, surface tension, and viscosity. TPC of choline chloride+citric acid and betaine+citric acid extracts were 8.75±0.17 and 7.47±0.04 mg gallic acid equivalents (GAE)/g of plant material, respectively. ABTS radical scavenging activity was 3.03±0.62 mmol Trolox/g of plant material (choline chloride+citric acid extract) and 2.82±0.13 mmol Trolox/g (betaine+citric acid extract), whereas, in DPPH assay, IC_{50} was 1.02±0.04 mg/mL for choline chloride+citric acid extract and 1.98±0.10 mg/mL for betaine+citric acid extract. The extraction yield was 0.575±0.035% (choline chloride+citric acid extract), and 0.490±0.016% (betaine+citric acid extract). pH values for chloride+citric acid and betaine+citric acid extracts were 0.83±0.03 and 2.68±0.02, while zeta potential was 2.46±0.16 and 0.88±0.09 mV, respectively. Conductivity for choline chloride+citric acid and betaine+citric acid extracts were 35.8±0.4 and 4.3±0.3 mS/cm, density was 1.16±0.01 and 1.54±0.02 g/mL, while surface tension was 35.2±0.8 and 37.2±0.1 mN/m, respectively. Viscosity was 6.52±0.01 mPa•s (choline chloride+citric acid extract) and 5.14±0.12 mPa•s (betaine+citric acid extract). Due to higher TPC and DPPH radical scavenging capacity, R. canina extract prepared using choline chloride and citric acid with 50% water in comparison to betaine+citric acid extract was favored as an ingredient in food, pharmaceutical, and cosmetic products.

Key words: *antioxidant activity, natural deep eutectic solvent, polyphenols, Rosa canina, viscosity.*

Introduction

Rosa canina L. (dog rose, Rosaceae) is frequently employed in traditional therapy, due to its diuretic, anti-inflammatory, anti-allergic, antioxidant, and analgesic activities. Dried rose hips are official in European Pharmacopoeia and frequently used in phytomedicine. Rosehips of *R. canina* are known for their preventive and curative effects against a wide range of renal, inflammatory, gout, and gastric disorders (Jafarirad et al., 2016). The fruits contain plenty of biologically active components, such as polyphenols, carotenoids, sugars, ascorbic acid, tocopherol, mineral elements, pectins, amino and fatty acids (Ouerghemmia et al., 2016; Veisi et al., 2016). The advantages of ultrasound-assisted extraction of the mentioned active principles include the increase of extraction yield and quality, fast kinetics, lower price, simple device, as well as a wide range of used extraction solvents (Jovanović et al., 2017). However, natural deep eutectic solvents (NADESs) are used with the aim to increase the release of polyphenols from the plant material and overcome the limitations of conventional and toxic organic solvents (Chemat et al., 2019; Hikmawanti et al., 2021). NADESs, as green solvents, are environmentally friendly, relatively safe, less hazardous, and biodegradable (Hikmawanti et al., 2021; Singh & Singh, 2018). Namely, NADESs contain compounds that are primary metabolites, such as sugars, plant acids, organic bases, and amino acids (Dai et al., 2013). Furthermore, an eutectic mixture can extract both hydrophilic and lipophilic molecules, whereas hydrophilic NADESs can dissolve some hydrophobic compounds, which is not the case with water (Hikmawanti et al., 2021).

In the present study, *R. canina* extracts were prepared using dried rose hips and two types of NADESs (choline chloride+citric acid with 50% of water and betaine+citric acid with 70% of water), and an ultrasound probe. The obtained extracts were characterized in terms of total polyphenol content (TPC), antioxidant potential (ABTS and DPPH assays), extraction yield, pH, zeta potential, conductivity, density, surface tension, and viscosity.

Materials and Methods

Plant material and reagents

R. canina pseudo fruits were from the Institute for Medicinal Plants Research "Dr Josif Pančić", Serbia. The following reagents were used: citric acid (Fisher Bioreagents, Belgium), betaine and choline chloride (Acros Organics, China), Folin-Ciocalteu reagent and gallic acid (Merck, Germany), sodium carbonate (Fisher Scientific, UK), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) - ABTS, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid - Trolox, and 2,2-diphenyl-1-picrylhydrazyl - DPPH (Sigma-Aldrich, USA), and ultrapure water.

Extraction

The extracts were obtained employing an ultrasound probe, Sonopuls (Bandelin, Germany) at 40% amplitude and two different NADESs (choline chloride+citric acid with 50% of water and betaine+citric acid with 70% of water), while the solid-to-solvent ratio was 1:100 g/mL and extraction time 10 min.

Measurement of total polyphenol content

The total polyphenol content (TPC) was determined spectrophotometrically at 765 nm using the modified Folin-Ciocalteu method (Galván d'Alessandro et al., 2012). The results are expressed as milligrams of gallic acid equivalents per gram of plant material (mg GAE/g).

Measurement of antioxidant activity (ABTS and DPPH assays)

The ABTS assay was based on the procedure described by Re et al. (1999) with a slight modification and the absorbance was measured at 734 nm. The antioxidant activity was expressed as mmol Trolox equivalent per g of plant material (mmol TE/g).
The DPPH assay was based on the procedure described by Horžić et al. (2009) with a slight modification and the absorbance was measured at 517 nm. The results were expressed as IC_{50} (mg/mL), defined as the concentration of the extract required to scavenge 50% of DPPH free radicals.

All spectrophotometric measurements were performed in an UV-1800 spectrophotometer (Shimadzu, Japan).

Determination of extraction yield

Extraction yield was calculated as:

$$EY\ (\%) = 100 - \frac{(a-b)*100}{m} \qquad \text{(Eq. 2)},$$

where *a* represents the weight (g) of the vessel containing the sample before drying, *b* represents the weight (g) of the vessel containing the sample after drying at 105ºC to constant mass, and *m* represents the weight (g) of the sample.

Measurement of pH, zeta potential, and conductivity

pH value of *R. canina* extracts was determined using pH meter HI 2211 (Hanna Instruments, USA). Each sample was measured three times at room temperature.

The measurements of zeta potential and conductivity were performed using photon correlation spectroscopy in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., UK). Each extract was measured three times at room temperature.

Measurement of density, surface tension, and viscosity

The density and surface tension of the extracts were determined using silicon crystal as the immersion body and Wilhelmy plate, respectively, in Force Tensiometer K20 (Kruss, Germany). Each extract (20 mL) was examined three times at room temperature.

The viscosity of the extracts was examined using Rotavisc lo-vi device equipment with VOL-C-RTD chamber, VOLS-1 adapter, and spindle (IKA, Germany). Each extract (6.7 mL) was examined three times at room temperature.

Statistical analysis

The statistical analysis was done by using analysis of variance (one-way ANOVA) and Duncan's *post hoc* test in STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

The influence of two types of NADESs (choline chloride+citric acid with 50% of water and betaine+citric acid with 70% of water) on TPC, antioxidant potential, extraction yield, zeta potential, conductivity, pH, density, surface tension, and viscosity of *R. canina* extracts were investigated and the results are shown in Table 1.

Table 1. Total polyphenol content (TPC), antioxidant activity (ABTS and DPPH methods), extraction yield (EY), zeta potential (ζ), conductivity (G), pH, density (ρ), surface tension (γ), and viscosity (η) of Rosa canina extracts obtained by using an ultrasound probe.

variables	choline chloride+citric acid+50% water	betaine+citric acid +70% water
TPC [mg GAE/g]	8.75±0.17[a*]	7.47±0.04[b]
ABTS [mmol TE/g]	3.03±0.62[a]	2.82±0.13[a]
DPPH IC_{50} [mg/mL]	1.02±0.04[a]	1.98±0.10[b]
EY [%]	0.575±0.035[a]	0.490±0.016[b]
ζ [mV]	2.46±0.16[a]	0.88±0.09[b]
G [mS/cm]	35.8±0.4[a]	4.3±0.3[b]
pH	0.83±0.03[b]	2.68±0.02[a]
ρ [g/mL]	1.16±0.01[b]	1.54±0.02[a]
γ [mN/m]	35.2±0.8[b]	37.2±0.1[a]
η [mPa·s]	6.52±0.01[a]	5.14±0.12[b]

**Values with different letters (a-b) in each row showed statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's post-hoc test); gallic acid equivalent, GAE; Trolox equivalent, TE; IC_{50}, the concentration of the extracts requires to neutralize 50% of free DPPH radicals.*

As can be seen in Table 1, there was a statistically significant difference between the TPC of the two prepared extracts. Namely, the TPC of choline chloride+citric acid extract was significantly higher in comparison to betaine+citric acid extract (8.75±0.17 and 7.47±0.04 mg GAE/g, respectively). According to Hikmawanti et al. (2021), NADESs can efficiently provide extracts of polyphenols with higher yields than those of conventional solvents. The difference between polyphenol yield in the extracts prepared using different NADESs, as well as different amounts of water, can be explained by the fact that the extraction process and the composition of the extraction solvent should be optimized for every herbal matrix (Jovanović et al., 2017). Furthermore, NADESs have two mechanisms of action: the interaction with target molecules through hydrogen bonding (direct) and the degradation of the cell wall, and consequently releasing of the target compounds from the plant matrix (Hikmawanti et al., 2021). A higher concentration of choline chloride and citric acid (and a lower amount of water) can cause the destabilization of plant cells and consequently provide a higher release of polyphenols in extraction medium in comparison to NADES with a higher amount of water.

However, ABTS radical scavenging activity was similar in both extracts, 3.03±0.62 mmol TE/g (choline chloride+citric acid extract) and 2.82±0.13 mmol TE/g (betaine+citric acid extract, Table 1). Namely, ABTS antioxidant activity did not follow the trend of TPC values. Various non-phenolic compounds, such as ascorbic acid, β-carotene, uric acid, triterpenoid saponins, thiols, and synergism of active compounds probably possess ABTS radical scavenging potential (Bi et al., 2012; Foti and Amorati, 2009). On the other hand, in the DPPH assay, IC_{50} was 1.02±0.04 mg/mL (significantly lower IC_{50}, i.e. higher antioxidant activity) for choline chloride+citric acid extract and 1.98±0.10 mg/mL for betaine+citric acid extract (Table 1), which was in correlation with TPC values. According to Hirano et al. (2001), the reduction of DPPH radicals may be due to the reducing property of flavonoids. Polyphenols and flavonoids can be effectively extracted using NADESs, but the content of individual compounds in extracts strongly depends on NADES composition (Hikmawanti et al., 2021).

The extraction yield was 0.575±0.035% (choline chloride+citric acid extract) and 0.490±0.016% (betaine+citric acid extract), while pH values for chloride+citric acid and betaine+citric acid extracts were 0.83±0.03 and 2.68±0.02 (Table 1). According to the literature data, the extraction yield strongly depends on the extraction time and extraction solvent (Rusak

et al., 2008). The extraction yield was significantly higher in choline chloride+citric acid extract as in the case of TPC value. Nevertheless, a higher extraction yield does not always correlate with the highest TPC and can be related to the recovery of ballast materials, including proteins, sugars, and lipids (Jovanović et al., 2021).

According to the results presented in Table 1, it can be concluded that the composition of NADES significantly affected the zeta potential of the extracts, as a measurement of the stability of the system. Indeed, choline chloride+citric acid extract had significantly higher zeta potential (2.46±0.16 mV) compared to betaine+citric acid extract (0.88±0.09 mV). Determination of the extract's zeta potential values is important from the aspect of its future application, including preparation of extract loaded-encapsulates or extract's use in coagulation and flocculation in drinking water and/or wastewater treatment. According to the literature data, the zeta potential of herbal extracts strongly depends on extraction conditions and extraction medium, varying from 2 mV to 15 mV (Skaf et al., 2021). The conductivity for choline chloride+citric acid and betaine+citric acid extracts were 35.8±0.4 and 4.3±0.3 mS/cm, respectively (Table 1). According to Jurinjak Tušek et al. (2018), the extracts with a higher conductivity value show better antioxidant activity, which was also the case with the DPPH radical scavenging activity of obtained *R. canina* extracts. However, in the case of the results of the ABTS assay, there was no statistically significant difference between extracts, while the conductivity of choline chloride+citric acid extract was significantly higher. It can be explained by the fact that ions from the eutectic medium can affect the conductivity, but not change the antioxidant potential of the extracts.

The density of choline chloride+citric acid and betaine+citric acid extracts was 1.16±0.01 and 1.54±0.02 g/mL, whereas surface tension was 35.2±0.8 and 37.2±0.1 mN/m, respectively. According to the literature data, the density is correlated to the extraction yield (Mladenović et al., 2018). However, it was not the case with the prepared *R. canina* extracts. On the other hand, Florindo et al. (2014) have reported that the density of eutectic solvent strongly depended on the composition and temperature. As can be seen from Table 1, *R. canina* extract with a higher water content (betaine+citric acid extract) possessed a higher surface tension, which can be explained by a relatively high interaction of water molecules through hydrogen bonds. Additionally, the decrease in surface tension can enhance the effect of diffusion and mass transfer (Peng et al., 2016) which was the case with chloride+citric acid extract (lower surface tension and higher polyphenol yield, Table 1). The viscosity of *R. canina* extracts with different NADESs varied from 6.52±0.01 mPa•s (choline chloride+citric acid extract) to 5.14±0.12 mPa•s (betaine+citric acid extract). The obtained results are expected since the increased water content in NADES (in betaine+citric acid extract) drastically decreased the extract's viscosity which was influenced by the strength of the hydrogen bonding and van der Walls interactions. According to the literature data, the viscosity of eutectic solvents was in the range from 0.05 to 50 mPa•s, while the composition of the extraction medium and temperature significantly influence the viscosity of the extracts (Abbott et al., 2004; Florindo et al., 2014).

Conclusions

The aim of the study was the development, physicochemical characterization, and investigation of the antioxidant potential of the two NADES extracts of *R. canina*. The choline chloride+citric acid *R. canina* extract (with a lower amount of water) possessed a higher TPC, extraction yield, zeta potential, conductivity, viscosity, and DPPH radical scavenging capacity. On the other hand, betaine+citric acid extract possessed higher density and surface tension. Therefore, the constitution of NADES should be optimized depending on the future application of the extract. However, due to higher TPC and DPPH radical scavenging activity, *R. canina* extract prepared using choline chloride and citric acid with 50% of water compared to betaine+citric acid extract with 70% of was favored as an ingredient in food, functional food, pharmaceutical, and cosmetic formulations.

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Conflict of interest

The authors declare that they have no financial and commercial conflicts of interest.

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