Natural deep eutectic solvent as tool for improving *Rosa canina* L. polyphenol recovery in maceration

ALEKSANDRA A. JOVANOVIĆ[1,*], RADA PJANOVIĆ[2], JELENA ŽIVKOVIĆ[3], DANICA ĆUJIĆ[1], MARIJA GNJATOVIĆ[1], SANDRA STEPANOVIĆ[1], AND KATARINA ŠAVIKIN[3]

[1] *University of Belgrade, Institute for the Application of Nuclear Energy INEP, Belgrade, Serbia*
[2] *University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia*
[3] *Institute for Medicinal Plants Research "Dr. Josif Pančić", Belgrade, Serbia*
[*] *Corresponding author: ajovanovic@inep.co.rs*

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Rosa canina* L. extracts were prepared using water or three natural deep eutectic solvents (NADESs: betaine+malic acid, betaine+sucrose, and citric acid+sucrose with 50% of water) and maceration. The extracts were characterized in terms of total polyphenol content (TPC), ABTS radical scavenging potential, extraction yield, zeta potential, conductivity, pH, density, surface tension, and viscosity. TPC was the highest in betaine+malic acid extract (10.4 mg gallic acid equivalents, GAE/g), and the lowest in water and citric acid+sucrose extracts (6.5 and 6.4 mg GAE/g, respectively). ABTS radical scavenging potential was the highest in water extract, 5.6 mmol Trolox/g, whereas the lowest was in citric acid+sucrose extract, 2.6 mmol Trolox/g. Extraction yield was the lowest for betaine+malic acid extract, 0.607 %, and statistically significantly higher for betaine+sucrose extract, 1.22 %. Zeta potential (absolute value) was the highest for betaine+sucrose extract (-2.12 mV), and the lowest for citric acid+sucrose extract (0.29 mV). Conductivity was in the range of 0.25 mS/cm (betaine+sucrose extract) to 5.46 mS/cm (betaine+malic acid extract). pH ranged from 3.0 in betaine+malic acid extract to 4.5 in water and betaine+sucrose extracts. Density varied from 1.00 g/mL for water extract to 1.19 g/mL for betaine+sucrose extract, while surface tension varied from 35.0 mN/m for betaine+sucrose extract to 40.6 mN/m for water extract. Viscosity of water extract was 1.52 mPa·s and it was significantly higher for citric acid+sucrose extract, 10.67 mPa·s. The application of NADESs as an extraction medium can improve polyphenol recovery from rose hips, as well as extraction yield and conductivity, but depending on NADES composition. Namely, the highest TPC and conductivity were measured in betaine+malic acid extract, while betaine+sucrose extract possesses the highest extraction yield. Thus, the constitution of NADES should be optimized depending on the future application of the extract.

Key words: extraction yield; maceration; natural deep eutectic solvent; polyphenols; rose hips

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1. INTRODUCTION

Rosa canina L. or dog rose (Rosaceae) is prickly bushes or shrubs native to Europe and Asia, as well as it is extensively cultivated over the world. *R. canina* represents the major commercial source of rose hips that are ellipsoid, globose, or ovoid fruits. The fruits of dog rose contains polyphenols (flavonoids, tannins, leucoanthocyanins, and phenolic acids), carotenoids, plant acids (citric and malic acids), minerals, vitamins (ascorbic acid and tocopherol), sugars (glucose, fructose, and sucrose), glycoproteins, glycolipids, oil, and pectin (Kazaz et al., 2009; Khan and Abourashed, 2010). Regarding to the pharmaceutical potential, antioxidant, anti-inflammatory, analgesic, mild laxative and diuretic activities of rose hips are reported (Khan and Abourashed, 2010; Larsen et al., 2003). Additionally, rose hips are mainly used as a source of acerola (natural vitamin C) that can be degraded during drying or extraction procedures. They are widely used as an herb tea ingredient and as a component of capsules and tablets (Khan and Abourashed, 2010).

The extraction of bioactive compounds, including polyphenols, carotenoids, alkaloids, terpenoids, proteins, sugars, steroids, and glycosides from different herbal materials represents an initial step to remove ballast substances and to isolate target components that can be used in food, pharmaceutical, and cosmetic industries, as well as in diagnostic procedures (Hikmawanti et al., 2021). Maceration represents a traditional

Table 1. Chemical composition, molar ratio, water content, and pH value of used natural deep eutectic solvents (NADESs).

NADES	molar ratio	water content	pH
betaine+malic acid	1:1	50 %	2.55
betaine+sucrose	1:1	50 %	4.35
citric acid+sucrose	1:1	50 %	2.06

procedure for obtaining plant extracts, but possesses several disadvantages, including lower extraction and polyphenol yields, extended extraction time, as well as a consumption of the large amounts of herbal material and extraction medium (Hikmawanti et al., 2021; Jovanović et al., 2017a). Thus, in the recent time, natural deep eutectic solvents (NADESs) are applied as a tool for improving polyphenol recovery using maceration. Furthermore, the use of relatively toxic organic solvents leads to improper extraction process, as well as to possibility of the presence of residual solvents in the final products. Also, volatile solvents are air pollutants that contribute to global warming (Chemat et al., 2019; Hikmawanti et al., 2021). Thus, the use of green solvents, such as NADESs that are environmentally friendly and relatively safe, could overcome the limitations of conventional organic solvents. The advantages of NADESs include environmentally friendly materials and procedures, easy preparation, less hazards, lower energy, and biodegradability (Hikmawanti et al., 2021; Promila and Singh, 2018). NADESs can be named natural mixtures due to their constituent components that are primary metabolites, including sugars, plant acids, organic bases, and amino acids (Dai et al., 2013). According to the literature, eutectic mixture can extract both hydrophilic and lipophilic molecules. Additionally, hydrophilic NADESs can dissolve some hydrophobic compounds, which is not the case with water as an extraction medium (Hikmawanti et al., 2021).
Therefore, in the present study, *R. canina* extracts prepared using water or natural deep eutectic solvents containing 50 % water, including two amino acids-based NADESs (betaine+malic acid and betaine+sucrose) and one neutral NADES with acids (citric acid+sucrose), and maceration, were characterized via analysing total polyphenol content (TPC), ABTS radical scavenging potential, extraction yield, zeta potential, conductivity, pH, density, surface tension, and viscosity.

2. MATERIALS AND METHODS

2.1. Plant material and reagents

Dried rose pseudo-fructus were obtained from the Institute for Medicinal Plants Research "Dr Josif Pančić", Serbia. The following reagents were used: ethanol and sodium carbonate (Fisher Scientific, UK), Folin-Ciocalteu reagent and gallic acid (Merck, Germany), potassium persulfate (Centrohem, Serbia), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS (Sigma-Aldrich), USA, betaine (Acros Organics, China), malic acid, sucrose, and citric acid (Fisher Bioreagents, Belgium).

2.2. NADESs and extracts preparation

Three NADESs (betaine+malic acid, betaine+sucrose, and citric acid+sucrose) were prepared in molar ratio 1:1 with 50 % of water at 80 °C until a transparent mixture was obtained (Mohd Fuad and Mohd Nadzir, 2022; Savi et al., 2019). Chemical composition, molar ratio, water content, and pH value of used NADESs are given in Table 1.

R. canina extract was obtained using maceration (the temperature of 25 °C was constant in the incubator shaker, KS 4000i control, IKA, Germany) for 60 min; grinded and sieved dried rose hip shells (0.75-2 mm, 0.35 g) and water or three types of NADESs (all with 50 % of water, 35 mL) are mixed and filtered after the extraction. The extracts were stored at 4 °C for further analyses.

2.3. Determination of total polyphenol content

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

2.4. Determination of ABTS radical scavenging activity

The absorbance of ethanol solution of ABTS radicals (2 mL) and diluted extract (20 µL) was spectrophotometrically measured at 734 nm after 6 min (Jovanović et al., 2021a), and calculated as:

$$\Delta A = A_0 - A_x\,,$$

where A_0 was the absorbance of ABTS solution and solvent; A_x was the absorbance of ABTS solution and extract. Scavenging capacity was expressed as mmol Trolox equivalents per gram of plant material (mmol Trolox/g).
The absorbance of all samples was read on UV spectrophotometer, UV-1800 (Shimadzu, Japan). Every spectrophotometric measurement was performed in triplicate.

2.5. Determination of extraction yield

Extraction yield was calculated as:

$$\mathrm{EY}\ (\%) = \frac{100 - ((a-b)\times 100)}{m}\,,$$

where a represents the weight (g) of vessel containing the sample prior to drying, b represents the weight (g) of vessel containing the sample after drying in Memmert 30-1060 (Memmert GmbH, Germany) at 105 °C to constant mass (approximately 4 h), and m represents the weight (g) of the sample.

2.6. Determination of pH, zeta potential, and conductivity

pH value of NADESs and *R. canina* extracts was determined using pH meter HI 2211 (Hanna Instruments, USA). Each sample was measured three times at room temperature. Zeta potential and conductivity of the extracts were determined by photon correlation spectroscopy (PCS) in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., UK). Each sample was measured three times at room temperature.

2.7. Determination of density, surface tension and viscosity

Density and surface tension of the extracts were measured using silicon crystal (as the immersion body) and Wilhelmy plate, respectively, in Force Tensiometer K20 (Kruss, Germany). Each sample was examined three times at room temperature. Viscosity of the extracts was determined using Rotavisc lo-vi device equipment with VOL-C-RTD chamber, VOLS-1 adapter and spindle (IKA, Germany). Each sample was examined three times at room temperature.

3. RESULTS AND DISCUSSION

Four different rose hips extracts, obtained using water and various NADESs containing 50 % of water (betaine+malic acid, betaine+sucrose, and citric acid+sucrose) and maceration, were examined in terms of physico-chemical characteristics (TPC and antioxidant potential) and physical characteristics

Table 2. Total polyphenol content (TPC), ABTS radical scavenging potential and extraction yield (EY) of *Rosa canina* L. extracts obtained using water or NADESs with 50 % water in maceration.

Extracts	TPC[a] [mg/g]	ABTS [mmol Trolox/g]	extraction yield [%]
water	6.5±0.4 c	5.6±0.5 a	0.654±0.04 c
betaine+malic acid (1:1)	10.4±0.5 a	4.1±0.1 b	0.607±0.09 d
betaine+sucrose (1:1)	9.1±0.1 b	3.9±0.3 b	1.220±0.05 a
citric acid+sucrose (2:1)	6.4±0.4 c	2.6±0.3 c	0.842±0.09 b

[a] Values with different letters (a-d) in each row showed statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's *post-hoc* test.

(extraction yield, zeta potential, conductivity, pH, density, surface tension, and viscosity).

3.1. Total polyphenol content of the extracts

As can be seen from Table 2, TPC was the highest in betaine+malic acid extract (10.4±0.5 mg GAE/g), followed by betaine+sucrose extract (9.1±0.1 mg GAE/g), and the lowest in water and citric acid+sucrose extracts (6.5±0.4 and 6.4±0.4 mg GAE/g, respectively).

According to the literature, NADESs are proven to efficiently provide extracts of plant metabolites with higher yields than those of conventional solvents, such are water, ethanol, methanol or their mixtures (Hikmawanti et al., 2021). For that reason better results of TPC in betaine based NADES extracts compared to water extract were expected (Table 2). The absence of better polyphenol yield in citric acid+sucrose extract can be explained by the fact that the extraction process, as well as the composition of the extraction medium should be optimized for every plant source (Jovanović et al., 2017b). Additionally, NADESs have two mechanisms of action: (1) direct action, i.e. the interaction with target molecules through hydrogen bonding and (2) indirect action, i.e. the damage of the cell wall, and consequently releasing of the target components from the plant material. In the second mechanism, NADESs have a role of pretreatment solvent, thus another extraction medium should be used in main extraction process (Hikmawanti et al., 2021). It can be the case with citric acid+sucrose, as an extraction medium. Namely, higher concentration of citric acid and sucrose can cause the destabilization of plant cells, but probably cannot provide superior leaking of polyphenols into extraction surrounding in comparison to water. Furthermore, NADESs based on amino acids, such as betaine, have higher polarity, while NADES with sugar components have a lower polarity (Dai et al., 2013). Therefore, it also can be the reason for better polyphenol recovery, as polar compounds, into extraction medium.

3.2. Antioxidant potential of the extracts

ABTS radical scavenging activity was the highest in water *R. canina* extract, 5.6±0.5 mmol Trolox/g of plant material, whereas the lowest was in citric acid+sucrose extract, 2.6±0.3 mmol Trolox/g (Table 2). Between betaine+malic acid and betaine+sucrose extracts there was no statistically significantly difference in antioxidant potential. It can be concluded that in the case of water and NADES *R. canina* extracts there was no correlation between TPC and antioxidant activity. According to the literature, various non-phenolic compounds, including ascorbic acid, β-carotene, uric acid, triterpenoid saponins, and thiols, as well as synergism of different components can have an important role in total antioxidant capacity of the plant extracts (Bi et al., 2012; Foti and Amorati, 2010). Probably, NADESs are suitable extraction medium for polyphenols of rose hips, but not for non-phenolic antioxidants, which show better diffusion through the aqueous medium. Also, NADES extracts can show the antioxidant potential due to intermolecular interactions, particularly hydrogen bonds, which stabilize the target molecules (Hikmawanti et al., 2021). According to Hikmawanti et al. (2021), polyphenols, flavonoids, even carotenoids can be effectively extracted using NADESs, but the content of individual compounds in extracts strongly depends on NADES composition. Additionally, *Eucalyptus globulus* extract obtained by using eutectic solvent did not increase polyphenol antioxidant capacity against DPPH radicals in comparison to aqueous-ethanolic extract (Gullón et al., 2019).

3.3. Extraction yield

Rusak et al. (2008) have reported that the extraction yield strongly depends on the extraction time and on the extraction medium as well. Thus, the mentioned parameter was measured in water and NADES extracts, and results are presented in Table 2. Extraction yield was the lowest for betaine+malic acid extract (0.607±0.09 %), while for betaine+sucrose extract it was statistically significantly higher than for other extracts (1.22±0.05 %). However, the highest extraction yield did not correlate with the highest TPC or antioxidant potential. On the contrary, the extract with the highest TPC (betaine+malic acid extract) possessed the lowest extraction yield. Namely, higher extraction yield can be correlated to the extraction of ballast materials, such are sugars, proteins or lipids (Jovanović et al., 2021b).

3.4. Zeta potential, conductivity, and pH value of the extracts

As can be seen from Table 3, zeta potential (absolute value), as a measurement of the stability of the system, was the highest for betaine+sucrose extract (-2.12±0.09 mV), and the lowest for citric acid+sucrose extract (0.29±0.03 mV). Measurement of zeta potential of the extracts is important from the aspect of the future application of the extract products, such as the preparation of extract loaded microparticles and nanoparticles, as well as for the potential use of the obtained extracts in coagulation and flocculation in drinking water treatment. Based on the obtained results, it can be noticed that the extracts prepared using betaine based NADESs exhibited negative, but higher zeta potential (absolute value), while water and citric acid+sucrose extract exhibited positive value of zeta potential. According to Skaf et al. (2021), zeta potential of plant extracts depends on extraction conditions and varies from 2 mV to 15 mV.

Conductivity, as a potential predictor of the antioxidant potential of the system, was in the range of 0.250±0.009 mS/cm

Table 3. Zeta potential (ζ), conductivity (G), acidity, density (ρ), surface tension (γ), and viscosity (η) of *Rosa canina* L. extracts obtained using water or NADESs with 50 % water in maceration.

Extracts	ζ[a] [mV]	G [mS/cm]	Acidity [pH]	ρ [g/mL]	γ [mN/m]	η [mPa/s]
water	0.43±0.03 c	1.39±0.34 c	4.5±0.0 a	1.00±0.04 c	40.6±1.2 a	1.52±0.01 d
betaine+malic acid (1:1)	-1.16±0.31 b	5.46±0.01 a	3.0±0.1 b	1.15±0.00 b	36.0±1.0 c	6.50±0.01 c
betaine+sucrose (1:1)	-2.12±0.09 a	0.25±0.01 d	4.5±0.1 a	1.19±0.00 a	35.0±1.04 c	9.50±0.01 b
citric acid+sucrose (1:1)	0.29±0.03 d	2.09±0.22 b	2.2±0.1 c	1.14±0.01 b	40.3±1.1 a	10.67±0.06 a

[a] Values with different letters (a-d) in each row showed statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's *post-hoc* test.

(betaine+sucrose extract) to 5.46±0.012 mS/cm (betaine+malic acid extract, Table 3). Abbott et al. (2004) have reported that NADESs' conductivities were in the range from 0.1 to 10 mS/cm changing with the composition and temperature. Furthermore, according to the literature, the extracts with higher conductivity possess the higher antioxidant potential (Jurinjak Tušek et al., 2018). However, it was not the case with NADESs *R. canina* extracts. It can be explained with the fact that ions from eutectic solvents influence the conductivity, but not improving the antioxidant activity of the extracts. Thus, water *R. canina* extract shows lower conductivity, but statistically significantly higher radical scavenging activity.

pH values ranged from 3.0 (betaine+malic acid extract) to 4.5 (water and betaine+sucrose extracts, Table 3). Namely, in all NADES extracts, pH values are quite increased in comparison to the initial extraction medium (Table 1), but they are still in acidic range of pH values. Although the pH values can affect the results of antioxidant test, in the case of ABTS assay , the mentioned influence is negligible. Namely, a very small amount of diluted extract (20 µL) was added in a large volume of ABTS solution (2 mL), thus it did not change the pH value of free radical solvent. Therefore, the measured pH values should be taken into account when choosing future encapsulation procedure or food, pharmaceutical, or cosmetic products for extract incorporation.

3.5. Density, surface tension, and viscosity of the extracts

Density varied from 1.00±0.01 g/mL for water extract to 1.19±0.01 g/mL for betaine+sucrose extract, while surface tension varied from 35.0±1.0 mN/m for betaine+sucrose extract to 40.6±1.2 mN/m for water extract (Table 3). Mladenović et al. (2018) have reported that density correlated to the extraction yield, which was the case with the obtained *R. canina* betaine+sucrose extract where the highest yield was detected. According to Florindo et al. (2014), density of eutectic solvent (cholinium chloride with different carboxylic acids) was 1.19 g/mL at room temperature, but depended on the composition and temperature. Regarding to the results of surface tension, *R. canina* water extract has a higher surface tension due to the relatively high interaction of water molecules through hydrogen bonds. According to Peng et al. (2016), the decrease in surface tension improves the effect of diffusion and mass transfer. It is in agreement with our results, where betaine+sucrose and betaine+citric acid extracts possess the lowest surface tension (Table 3) and the highest polyphenol recovery (Table 2).

The major disadvantage of NADES in comparison to conventional solvents, including water, ethanol and their mixture, is inherent high viscosity that can reduce the diffusion coefficients of analytes causing a slow mass transfer and extended extraction time. Thus, the addition of a certain amount of water in eutectic mixture can overcome the mentioned problem and improve the yield of target compounds (Dai et al., 2013; Hikmawanti et al., 2021). The increased water content in NADES drastically decreases the viscosity that strictly depends on the strength of the hydrogen bonding and van der Walls interactions. Namely, the stronger hydrogen bonding is make the extraction medium more viscous, and consequently causes a lower solubility of the active components (Hikmawanti et al., 2021; Mehariya et al., 2021). Additionally, the implementation of very viscous extracts, such as NADES samples, in food, pharmaceutical, and cosmetic preparations can cause various technical difficulties. Therefore, viscosity of water and NADES extracts from rose hips are examined. Viscosity was the lowest for water extract, 1.52±0.01 mPa·s, whereas it was statistically significantly higher for citric acid+sucrose extract, 10.67±0.06 mPa·s (Table 3). Hassan and Hobani (1998) have also reported that water extract of *Hibiscus sabdariffa* showed viscosity of 1.5 mPa·s. The viscosity values of eutectic solvents were found to cover the range from 0.05 to 50 mPa·s (Abbott et al., 2004; Florindo et al., 2014). The viscosity depends on NADES composition and temperature as well (Abbott et al., 2004; Florindo et al., 2014; Ruesgas-Ramón et al., 2017). Since the viscosity is related to the free volume and the probability of finding holes of suitable dimensions for the molecules or ions transfer into vacant sites, the decrease in surface tension causes the increase in free volume and thus the decrease in extraction medium viscosity (Ruesgas-Ramón et al., 2017). As can be seen in Table 3, the NADES sample with higher surface tension (citric acid+sucrose extract) possessed higher value of viscosity compared to other NADES extracts.

CONCLUSION

In the present study, water extract and three NADES extracts of *R. canina* were prepared and their physico-chemical properties were investigated. The highest TPC and conductivity were measured in betaine+malic acid extract, while betaine+sucrose extract possesses the highest extraction yield. On the other hand, water extract exerted the highest ABTS radical scavenging activity. Betaine based NADES extracts showed the lowest surface tension, and thus higher polyphenol recovery, while citric acid+sucrose extract possessed higher surface tension and consequently the highest viscosity. The application of NADESs as an extraction medium can improve polyphenol recovery from rose hips, as well as extraction yield and conductivity, but depending on NADES composition. Thus, the constitution of NADES should be optimized depending on the future application of the extract.

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REFERENCES

Abbott, A. P., Boothby, D., Capper, G., Davies, D. L. and Rasheed, R. K. (2004). Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids, *Journal of the American Chemical Society* **126**(29): 9142–9147.

Bi, L., Tian, X., Dou, F., Hong, L., Tang, H. and Wang, S. (2012). New antioxidant and antiglycation active triterpenoid saponins from the root bark of *Aralia taibaiensis*, *Fitoterapia* **83**(1): 234–240.

Chemat, F., Abert-Vian, M., Fabiano-Tixier, A. S., Strube, J., Uhlenbrock, L., Gunjevic, V. and Cravotto, G. (2019). Green extraction of natural products. Origins, current status, and future challenges, *TrAC Trends in Analytical Chemistry* **118**: 248–263.

Dai, Y., van Spronsen, J., Witkamp, G.-J., Verpoorte, R. and Choi, Y. H. (2013). Natural deep eutectic solvents as new potential media for green technology, *Analytica Chimica Acta* **766**: 61–68.

Florindo, C., Oliveira, F. S., Rebelo, L. P. N., Fernandes, A. M. and Marrucho, I. M. (2014). Insights into the synthesis and properties of deep eutectic solvents based on cholinium chloride and carboxylic acids, *ACS Sustainable Chemistry & Engineering* **2**(10): 2416–2425.

Foti, M. C. and Amorati, R. (2010). Non-phenolic radical-trapping antioxidants, *Journal of Pharmacy and Pharmacology* **61**(11): 1435–1448.

Galvan d'Alessandro, L., Kriaa, K., Nikov, I. and Dimitrov, K. (2012). Ultrasound assisted extraction of polyphenols from black chokeberry, *Separation and Purification Technology* **93**: 42–47.

Gullón, B., Muñiz-Mouro, A., Lú-Chau, T. A., Moreira, M. T., Lema, J. M. and Eibes, G. (2019). Green approaches for the extraction of antioxidants from eucalyptus leaves, *Industrial Crops and Products* **138**: 111473.

Hassan, B. and Hobani, A. (1998). Flow properties of Roselle (*Hibiscus sabdariffa* L.) extract, *Journal of Food Engineering* **35**(4): 459–470.

Hikmawanti, N. P. E., Ramadon, D., Jantan, I. and Mun'im, A. (2021). Natural deep eutectic solvents (NADES): Phytochemical extraction performance enhancer for pharmaceutical and nutraceutical product development, *Plants* **10**(10): 2091.

Jovanović, A. A., Djordjević, V. B., Petrović, P. M., Pljevljakušić, D. S., Zdunić, G. M., Šavikin, K. P. and Bugarski, B. M. (2021a). The influence of different extraction conditions on polyphenol content, antioxidant and antimicrobial activities of wild thyme, *Journal of Applied Research on Medicinal and Aromatic Plants* **25**: 100328.

Jovanović, A. A., Lević, S. M., Pavlović, V. B., Marković, S. B., Pjanović, R. V., Đorđević, V. B., Nedović, V. and Bugarski, B. M. (2021b). Freeze vs. Spray drying for dry wild thyme (Thymus serpyllum L.) extract formulations: The impact of gelatin as a coating material, *Molecules* **26**(13): 3933.

Jovanović, A. A., Đorđević, V. B., Zdunić, G. M., Pljevljakušić, D. S., Šavikin, K. P., Gođevac, D. M. and Bugarski, B. M. (2017a). Optimization of the extraction process of polyphenols from *Thymus serpyllum* L. herb using maceration, heat- and ultrasound-assisted techniques, *Separation and Purification Technology* **179**: 369–380.

Jovanović, A., Petrović, P., Đordjević, V., Zdunić, G., Šavikin, K. and Bugarski, B. (2017b). Polyphenols extraction from plant sources, *Lekovite sirovine* **37**: 45–49.

Jurinjak Tušek, A., Benković, M., Valinger, D., Jurina, T., Belščak-Cvitanović, A. and Gajdoš Kljusurić, J. (2018). Optimizing bioactive compounds extraction from different medicinal plants and prediction through nonlinear and linear models, *Industrial Crops and Products* **126**: 449–458.

Kazaz, S., Baydar, H. and Erbas, S. (2009). Variations in chemical compositions of *Rosa damascena* Mill. and *Rosa canina* L. fruits, *Czech Journal of Food Sciences* **27**(No. 3): 178–184.

Khan, I. A. and Abourashed, E. A. (2010). *Leung's Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*, John Wiley & Sons, Inc., Hoboken, New Jersey.

Larsen, E., Kharazmi, A., Christensen, L. P. and Christensen, S. B. (2003). An antiinflammatory galactolipid from rose hip (*Rosa canina*) that inhibits chemotaxis of human peripheral blood neutrophils *in vitro*, *Journal of Natural Products* **66**(7): 994–995.

Mehariya, S., Fratini, F., Lavecchia, R. and Zuorro, A. (2021). Green extraction of value-added compounds form microalgae: A short review on natural deep eutectic solvents (NaDES) and related pre-treatments, *Journal of Environmental Chemical Engineering* **9**(5): 105989.

Mladenović, J., Đurić, M., Šekularac, G., Brković, D., Stepanović, J., Mašković, P. and Bošković-Rakočević, L. (2018). Determination of the content of bioactive components in different extracts of *Portulaca oleracea* L., *Acta agriculturae Serbica* **23**(46): 223–231.

Mohd Fuad, F. and Mohd Nadzir, M. (2022). The formulation and physicochemical properties of betaine-based natural deep eutectic solvent, *Journal of Molecular Liquids* **360**: 119392.

Peng, X., Duan, M.-H., Yao, X.-H., Zhang, Y.-H., Zhao, C.-J., Zu, Y.-G. and Fu, Y.-J. (2016). Green extraction of five target phenolic acids from *Lonicerae japonicae flos* with deep eutectic solvent, *Separation and Purification Technology* **157**: 249–257.

Promila and Singh, S. (2018). Applications of green solvents in extraction of phytochemicals from medicinal plants: A review, *The Pharma Innovation Journal* **7**(3): 238–245. Publisher: AkiNik Publications.

Ruesgas-Ramón, M., Figueroa-Espinoza, M. C. and Durand, E. (2017). Application of deep eutectic solvents (des) for phenolic compounds extraction: overview, challenges, and opportunities, *Journal of Agricultural and Food Chemistry* **65**(18): 3591–3601.

Rusak, G., Komes, D., Likić, S., Horžić, D. and Kovač, M. (2008). Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used, *Food Chemistry* **110**(4): 852–858.

Savi, L. K., Dias, M. C. G. C., Carpine, D., Waszczynskyj, N., Ribani, R. H. and Haminiuk, C. W. I. (2019). Natural deep eutectic solvents (NADES) based on citric acid and sucrose as a potential green technology: A comprehensive study of water inclusion and its effect on thermal, physical and rheological properties, *International Journal of Food Science & Technology* **54**(3): 898–907.

Skaf, D. W., Punzi, V. L., Rolle, J. T. and Cullen, E. (2021). Impact of *Moringa oleifera* extraction conditions on zeta potential and coagulation effectiveness, *Journal of Environmental Chemical Engineering* **9**(1): 104687.