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Innovative Green Extraction of Phenolic Compounds from Chicory (*Cichorium Intybus*) Using Natural Deep Eutectic Solvents

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Abstract

Introduction: Chicory (*Cichorium intybus* L.) exhibits antioxidant, antibacterial, antidiabetic, and anti-inflammatory properties, owing to its bioactive components, with flavonoids and phenolic acids being the most significant. Natural deep eutectic solvents (NADES) are increasingly used as an alternative to conventional solvents in the extraction of active compounds, thanks to their non-toxicity.

Goal: To investigate the efficiency of flavonoid and phenolic acid ultrasound extraction from chicory herb and root using NADES and to compare the results with extracts obtained using conventional solvents–70% ethanol, methanol, and water.

Material and methods: A total of sixteen samples were examined, eight samples of chicory herb and eight samples of chicory root. Extraction was performed using five different NADES, 70% ethanol, methanol, and water. NADES were prepared by mixing and heating. The content of phenolic acids and flavonoids was determined using high-performance liquid chromatography (HPLC).

Results: Ten bioactive compounds were identified and quantified in the analyzed extracts: phenolic acids gallic acid, chlorogenic acid, para-hydroxybenzoic acid, caffeic acid, coumaric acid, ferulic acid, rosmarinic acid and epicatechin quercetin, and naringenin from flavonoids. In eight out of ten extracts obtained using NADES, the content of bioactive compounds was significantly higher compared to extracts obtained using conventional solvents–70% ethanol, methanol, and water.

Conclusion: In this study, the efficiency of extracting phenolic acids and flavonoids from the root and herb of chicory using NADES was analysed and confirmed. The use of novel green solvents resulted in significantly higher yields of phenolic compounds compared to conventional solvents.

Keywords

NADES, chicory, ultrasound extraction, flavonoids, phenolic acids

1 Introduction

Chicory (*Cichorium intybus* L.), a member of the Asteraceae family, has been recognized since antiquity for its medicinal properties and remains relevant in contemporary medical applications [1, 2]. It exhibits multifaceted biological activities, including modulation of metabolism, and possesses antioxidant, antimicrobial, antifungal, antidiabetic, and analgesic effects. Furthermore, it has shown hepatoprotective and choleprotective potential and may serve as a bioindicator of heavy metals such as lead, cadmium, copper, and zinc in the human body [3, 4].

Chicory is rich in various secondary metabolites–phenolic acids, fatty acids, flavonoids, triterpenoids, sesquiterpene lactones, and amino acids–as well as essential nutrients and minerals, including vitamins B1, B2, C, β-carotene, magnesium, potassium, sodium, and manganese [3]. Its root is particularly valued for its prebiotic properties, promoting calcium absorption and demonstrating efficacy in the prevention of atherosclerosis. Additionally, it contributes to lowering the glycemic index of foods and helps regulate blood lipid profiles, making it increasingly utilized in the dietary management of diabetes [5].

Chicory has demonstrated antiviral activity against Herpes simplex virus type 1 (HSV-1) and adenovirus [1]. Due to its high content of caffeic acid, a compound with notable antiviral properties, it is hypothesized that chicory may exert beneficial effects against SARS-CoV-2, although

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further investigation is required [6]. Chicoric acid, another bioactive constituent, has been shown to inhibit HIV infection and the enzyme hyaluronidase, thereby potentially impeding viral DNA replication and offering hepatocyte protection against duck hepatitis B virus (DHBV) [7].

In animal studies, methanolic extracts of chicory have reduced serum levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), indicating a hepatoprotective effect and reduced mortality in mice subjected to acetaminophen-induced liver injury [8]. Similarly, in male mice, chicory mitigated hepatotoxicity and oxidative stress induced by nitrosamine precursors (chlorpromazine and sodium nitrite), likely due to its radical-scavenging capacity [9].

Chicory exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. Methanolic and aqueous extracts of aerial parts have demonstrated superior antimicrobial efficacy at times exceeding that of standard antibiotics against pathogens such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*. Aqueous extracts of chicory seeds also showed pronounced activity against *S. aureus*, surpassing that of the aerial parts. In addition, growth inhibition was observed for *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, and *Salmonella typhi* [10–12].

Beyond its therapeutic roles, chicory is also employed as a coffee substitute and as livestock feed [13]. However, the use of conventional organic solvents such as methanol and ethanol in extraction processes has raised environmental and safety concerns. These solvents are associated with issues including low extraction yield due to thermal degradation, flammability, and ecological toxicity, prompting the scientific community to seek greener alternatives [14].

Deep eutectic solvents (DES) have emerged as promising eco-friendly extraction media. These systems consist of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) that, upon mixing under specific conditions, form a eutectic mixture with a melting point lower than that of the individual components [15]. DES are biodegradable, sustainable, and cost-effective [16].

A notable subclass of DES is natural deep eutectic solvents (NADES), composed of naturally occurring biomolecules such as amino acids, sugars, carboxylic acids, choline chloride, and urea. The physicochemical properties of these solvents including density, viscosity, melting point, polarity, pressure, and pH are key determinants of their specific applications [17].

Depending on their composition, NADES can solubilize both hydrophilic and hydrophobic compounds. For instance, those containing organic acids display the highest polarity, followed by amino acid-based mixtures, whereas sugar and polyol-based NADES are the least polar [18]. A major limitation of NADES is their high viscosity, which can impede extraction efficiency and process kinetics. Notably, solvents composed of choline chloride and sugar exhibit the highest viscosity, whereas polyol-based systems are relatively less viscous [19]. This is largely attributed to the dense network of hydrogen bonds between HBA and HBD components. Increasing the temperature or adding water can partially alleviate this challenge [20].

NADES are increasingly utilized for the extraction of bioactive compounds from a wide range of natural sources, offering a sustainable alternative for harnessing the full therapeutic potential of medicinal plants such as *Cichorium intybus* [21]. The combination of ultrasound-assisted extraction (UAE) with NADES offers several advantages. Ultrasound facilitates cell wall disruption and enhances mass transfer, thereby reducing extraction time and solvent consumption, while NADES provide high solubilization capacity for diverse phenolic compounds, combined with biodegradability and low toxicity. This synergy results in improved extraction yields and preservation of thermolabile compounds. However, certain limitations should be acknowledged, such as the relatively high viscosity of NADES, which can reduce diffusivity and complicate downstream processing. In addition, optimization of water content and ultrasound parameters is often required to balance extraction efficiency and compound stability. Despite these challenges, UAE–NADES has emerged as a promising green alternative to conventional solvent extraction, as demonstrated in various plant matrices [22–24].

The aim of this study is to evaluate the efficiency of bioactive compound extraction from the aerial parts and roots of *Cichorium intybus* using NADES in combination with UAE as an innovative and sustainable technique. Furthermore, the study seeks to compare the extraction outcomes with those obtained using conventional solvents 70% ethanol, methanol, and water to assess the potential of NADES as a natural and safer alternative to hazardous organic solvents.

2 Materials and methods

The materials utilized in this study included both conventional and natural solvents. Conventional organic solvents ethanol (J.T. Baker, Netherlands) and methanol (POCH, Poland) were used alongside water and five

types of NADES. The NADES were prepared from analytical-grade components: D-(+)-glucose (J.T. Baker), citric acid monohydrate (Lach:ner), glycerol (Lach:ner), urea (Centrohem), and lactic acid (Centrohem).

Plant materials dried herbal and root teas of Cichorium intybus were sourced from the Institute for Medicinal Plant Research "Dr. Josif Pančić" in Belgrade, Serbia (herb batch no. 00920123; root batch no. 01761023).

2.1 Sample preparation

The dried aerial parts and roots of chicory were purchased from a local pharmacy in Novi Sad, Serbia. Both were ground using a laboratory mill and sieved through a 0.3 mm mesh. Precisely 0.2000 g of each powdered sample was weighed using an analytical balance (KERN & SOHN GmbH, Germany) and transferred into test tubes for extraction.

2.2 Preparation of NADES

Five NADES were synthesized by combining selected HBAs and donors at specific molar ratios:

- Glycerol and citric acid (2:1): produced a transparent, light-yellow, semi-viscous liquid (NADES 1 – Gly/Cit).
- Glycerol and urea (1:1): yielded a clear, colorless liquid (NADES 2 – Gly/Urea).
- Glucose and urea (1:2): resulted in a transparent, semi-viscous, colorless liquid (NADES 3 – Glu/Urea).
- Glucose and lactic acid (1:5): formed a clear, colorless solution (NADES 4 – Glu/Lac).
- Glycerol and glucose (2:1): yielded a clear, colorless solvent (NADES 5 – Gly/Glu).

Each solvent was prepared by heating the component mixture on a hot plate with magnetic stirring (300 rpm) at 50–80 °C for 1 h. The resulting eutectic mixtures were then diluted with distilled water in a 5:1 ratio to obtain 20% aqueous NADES, which were subsequently added to the plant material in the test tubes.

2.3 Extraction procedure

Each 0.2000 g sample of plant material was treated with 5 mL of solvent: either one of the five NADES (4 mL of NADES + 1 mL water), or with 5 mL of a conventional solvent 70% ethanol, methanol, or distilled water. All test tubes were subjected to UAE in an ultrasonic bath (VAB SB 6 LT; Vabsonic, Serbia) for 20 min at 35 kHz, at ambient temperature (25 ± 2 °C). In the case of NADES-based extracts. Following sonication, the samples were centrifuged at 3500 rpm for 15 min using a Sigma centrifuge (Germany).

The resulting supernatant from each sample was collected using a micropipette and transferred into beakers. For NADES samples, methanol was added to each sample prior to filtration to decrease the viscosity of the solvent system and improve filterability, as well as to ensure compatibility with the high-performance liquid chromatography (HPLC) mobile phase. All extracts were filtered through RC-45/25 membrane filters (0.45 μm) and analyzed by HPLC.

2.4 HPLC

The HPLC method was adapted from Miljić et al. with modifications [25]. Validation was performed according to ICH guidelines. Calibration curves for all phenolic standards exhibited excellent linearity ($R^2 > 0.995$). Limits of detection (LOD) ranged from 0.02 to 0.15 μg/mL, while limits of quantification (LOQ) were in the range 0.05–0.45 μg/mL. Recovery values obtained in conventional matrices ranged from 93–106% for representative standards (gallic acid, chlorogenic acid, quercetin). Given the potential viscosity-related matrix effects of NADES extracts, recovery testing was conducted with dilution and methanol addition steps to minimize such interferences. Chromatographic separation was performed on a Dionex system (Thermo Fisher Scientific, UK) equipped with a Zorbax Eclipse C18 column (4.6 mm × 150 mm, 5 μm particle size; Agilent Technologies, USA). The mobile phase consisted of 0.1% acetic acid in deionized water (solvent A) and 0.1% acetic acid in acetonitrile (solvent B), with a gradient elution profile:

- 0–3.25 min: 10% B
- 3.25–8.00 min: 12% B
- 8.00–15.00 min: 25% B
- 15.00–15.80 min: 30% B
- 15.80–25.00 min: 90% B
- 25.00–25.40 min: 100% B

The flow rate was maintained at 1.0 mL/min, and the injection volume was 5 μL. The column was held at 25 °C. All phenolic compounds were quantified at 280 nm. Although some compounds, such as rosmarinic acid, exhibit absorption maxima at higher wavelengths (e.g., ~330 nm), 280 nm was selected as a compromise wavelength to enable simultaneous quantification of multiple phenolics within complex chicory extracts. This

approach has been widely applied in phenolic profiling studies and provides consistent and comparable results across compound classes. Each analysis lasted 30 min.

2.5 Statistical analysis

Results are presented as mean values ± standard deviation. Statistical evaluation was performed using IBM SPSS Statistics v.26. One-way analysis of variance (ANOVA) followed by Duncan's post hoc test was used to assess differences between samples. A p-value < 0.05 was considered statistically significant.

3 Results and discussion

A total of ten bioactive compounds were identified and quantified in the extracts obtained from both the root, Table 1 and aerial parts Table 2 of *Cichorium intybus* using five NADES and three conventional solvents (methanol, ethanol, and water). The identified compounds included seven phenolic acids: gallic, chlorogenic, para-hydroxybenzoic, caffeic, coumaric, ferulic, and rosmarinic acid and three flavonoids: epicatechin, quercetin, and naringenin.

In the root extracts (Table 1), the concentrations of gallic acid ranged from 0.32 mg/g (in conventional solvents) to 4.23 mg/g (using glycerol/urea NADES). Chlorogenic acid content was highest in the glucose/lactic acid NADES (5.69 mg/g), while caffeic acid was consistently extracted in high concentrations (up to 5.06 mg/g) across all NADES. Epicatechin was best extracted using glucose/glucose-based solvents (up to 3.76 mg/g), and quercetin and naringenin were also significantly more abundant in NADES extracts compared to conventional solvents.

In the aerial part extracts (Table 2), phenolic acid concentrations were generally higher than those in root extracts. For instance, chlorogenic acid reached 18.13 mg/g in the glucose/urea system–approximately 70 times higher than in the water extract. Caffeic acid concentration peaked at 14.18 mg/g using glycerol/glucose NADES. Para hydroxybenzoic acid showed the highest yield with

Table 1 Quantitative content of phenolic compounds (mg/g) extracted by NADES and conventional solvents from *C. intybus* roots*

Active principles	Gly/Cit	Gly/Urea	Glu/Urea	Glu/Lac	Gly/Glu	Ethanol	Methanol	Water
Gallic acid	3.18 ± 0.15^{a}	4.23 ± 0.20^{b}	3.48 ± 0.20^{c}	$3.32 \pm 0.20^{a,c}$	$3.32 \pm 0.20^{a,c}$	0.34 ± 0.03^{d}	0.32 ± 0.02^{d}	0.33 ± 0.02^{d}
Chlorogenic acid	2.68 ± 0.20^{a}	0.94 ± 0.03^{b}	1.10 ± 0.15^{b}	5.69 ± 0.20^{c}	2.48 ± 0.20^{a}	0.95 ± 0.03^{b}	$0.92 \pm 0,02^{b}$	0.32 ± 0.02^{d}
p-Hydroxi-benzoic acid	nd**	0.33 ± 0.02^{a}	1.90 ± 0.10^{b}	0.06 ± 0.003^{c}	4.72 ± 0.20^{d}	0.09 ± 0.004^{c}	0.004 ± 0.002^{c}	0.44 ± 0.01^{a}
Caffeic acid	4.78 ± 0.20^{a}	5.06 ± 0.15^{b}	$4.90 \pm 0.20^{a,b}$	$4.83 \pm 0.15^{a,b}$	5.05 ± 0.15^{b}	0.74 ± 0.01^{c}	0.61 ± 0.01^{c}	0.85 ± 0.02^{c}
Epicatechin	3.08 ± 0.15^{a}	3.14 ± 0.15^{a}	3.47 ± 0.20^{b}	3.06 ± 0.10^{a}	3.76 ± 0.20^{c}	0.32 ± 0.01^{d}	0.46 ± 0.02^{d}	0.32 ± 0.02^{d}
Coumaric acid	0.34 ± 0.02^{a}	nd	0.35 ± 0.19^{a}	0.32 ± 0.02^{a}	0.60 ± 0.01^{b}	0.08 ± 0.01^{c}	0.03 ± 0.01^{d}	0.11 ± 0.01^{e}
Ferulic acid	3.52 ± 0.10^{a}	3.20 ± 0.20^{b}	3.18 ± 0.15^{b}	3.21 ± 0.15^{b}	3.20 ± 0.15^{b}	0.51 ± 0.02^{c}	0.42 ± 0.01^{c}	0.50 ± 0.02^{c}
Rosmarinic acid	nd	0.21 ± 0.01^{a}	1.07 ± 0.15^{b}	0.64 ± 0.02^{c}	2.73 ± 0.15^{d}	0.39 ± 0.01^{e}	0.16 ± 0.01^{a}	0.10 ± 0.01^{a}
Quercetin	1.47 ± 0.12^{a}	2.15 ± 0.15^{b}	2.32 ± 0.15^{b}	1.66 ± 0.10^{a}	3.27 ± 0.20^{c}	0.33 ± 0.02^{d}	0.52 ± 0.01^{d}	0.35 ± 0.01^{d}
Naringenin	3.69 ± 0.10^{a}	3.67 ± 0.15^{a}	3.70 ± 0.10^{a}	3.70 ± 0.10^{a}	3.92 ± 0.12^{b}	0.41 ± 0.01^{c}	0.40 ± 0.02^{c}	0.39 ± 0.02^{c}

*Different superscript letters within the same active compound indicate statistically significant differences among samples.
**nd: not detected (sample concentration lower than that which can be detected using laboratory methods).

Table 2 Quantitative content of phenolic compounds (mg/g) extracted by NADES and conventional solvents from the herb *C. intybus**

Active principles	Gly/Cit	Gly/Urea	Glu/Urea	Glu/Lac	Gly/Glu	Ethanol	Methanol	Water
Gallic acid	3.68 ± 0.22^{a}	$3.58 \pm 0.15^{a,b}$	4.30 ± 0.10^{c}	3.38 ± 0.10^{b}	7.43 ± 0.10^{d}	0.38 ± 0.01^{e}	1.14 ± 0.10^{f}	0.35 ± 0.02^{e}
Chlorogenic acid	8.45 ± 0.24^{a}	2.54 ± 0.25^{b}	18.13 ± 1.00^{c}	4.35 ± 0.25^{d}	4.27 ± 0.15^{d}	3.00 ± 0.20^{b}	3.71 ± 0.15^{d}	0.26 ± 0.02^{e}
p-Hydroxi- benzoic acid	0.95 ± 0.03^{a}	2.90 ± 0.30^{b}	4.52 ± 0.20^{c}	1.61 ± 0.15^{d}	9.22 ± 0.21^{e}	1.09 ± 0.15^{a}	1.02 ± 0.10^{a}	0.22 ± 0.02^{f}
Caffeic acid	4.90 ± 0.29^{a}	5.89 ± 0.25^{b}	$5.27 \pm 0.31^{a,b}$	4.95 ± 0.15^{a}	14.18 ± 1.00^{c}	4.59 ± 0.30^{a}	2.12 ± 0.25^{d}	0.63 ± 0.02^{e}
Epicatechin	3.62 ± 0.24^{a}	4.50 ± 0.25^{b}	4.86 ± 0.20^{b}	3.58 ± 0.30^{a}	$4.67 \pm 0,25^{b}$	0.51 ± 0.02^{c}	1.59 ± 0.20^{d}	0.59 ± 0.02^{c}
Coumaric acid	0.45 ± 0.01^{a}	0.43 ± 0.02^{a}	1.31 ± 0.25^{b}	2.52 ± 0.20^{c}	2.57 ± 0.25^{c}	0.97 ± 0.02^{d}	0.23 ± 0.02^{a}	0.22 ± 0.01^{a}
Ferulic acid	3.36 ± 0.20^{a}	4.39 ± 0.25^{b}	4.38 ± 0.25^{b}	3.37 ± 0.20^{a}	3.65 ± 0.25^{a}	1.21 ± 0.21^{c}	0.73 ± 0.34^{d}	0.73 ± 0.03^{d}
Rosmarinic acid	nd**	0.31 ± 0.02^{a}	0.47 ± 0.03^{b}	0.31 ± 0.02^{a}	1.68 ± 0.10^{c}	0.84 ± 0.41^{d}	0.61 ± 0.36^{e}	0.58 ± 0.24^{e}
Quercetin	2.62 ± 0.20^{a}	1.71 ± 0.12^{b}	$1.52 \pm 0.20^{b,c}$	2.69 ± 0.20^{a}	2.60 ± 0.25^{a}	1.37 ± 0.20^{c}	1.04 ± 0.10^{d}	0.41 ± 0.01^{e}
Naringenin	3.79 ± 0.20^{a}	3.78 ± 0.20^{a}	3.80 ± 0.21^{a}	3.77 ± 0.20^{a}	3.76 ± 0.20^{a}	0.42 ± 0.21^{b}	0.46 ± 0.03^{b}	0.52 ± 0.01^{b}

*Different superscript letters within the same active compound indicate statistically significant differences among samples.
**nd: not detected (sample concentration lower than that which can be detected using laboratory methods).

glycerol/glucose (9.22 mg/g). Similarly, the content of epicatechin, quercetin, and naringenin was significantly elevated in all NADES-based extracts compared to conventional solvents. In contrast, rosmarinic and coumaric acids were found at lower concentrations across all samples.

Overall, in 8 out of 10 comparisons, NADES resulted in significantly higher yields of phenolic and flavonoid compounds than standard solvents. Statistical analysis ($p < 0.05$) confirmed the superior extraction efficiency of NADES for most bioactive components.

In this study, the efficiency of NADES in combination with UAE was evaluated. Quantitative analysis was performed to assess the extraction efficiency of bioactive compounds from the aerial parts and roots of chicory. The concentrations of phenolic acids and flavonoids obtained using NADES were compared to those extracted using conventional solvents, including methanol, ethanol, and water.

The dominant compounds identified included chlorogenic, caffeic, and gallic acids, followed by ferulic acid, epicatechin, and naringenin. In smaller amounts, para-hydroxybenzoic acid, quercetin, coumaric acid, and rosmarinic acid were also extracted. These compounds are particularly valued for their antioxidant properties, contributing to the scavenging of free radicals and protection against oxidative stress. Additionally, they exhibit anti-inflammatory, antipyretic, antibacterial, and antidiabetic activities [3].

NADES, particularly those based on glycerol/citric acid, glycerol/urea, and glucose/lactic acid, achieved gallic acid concentrations up to 12 times higher than conventional solvents, while root extractions were approximately tenfold more efficient. Glucose/urea was the most effective NADES for chlorogenic acid, yielding a 70-fold increase over water. Glycerol/urea and glucose/urea significantly enhanced para-hydroxybenzoic acid extraction from the aerial parts, with glycerol/glucose being the only system effective in root extractions. Caffeic acid was best extracted using glycerol/glucose, and all NADES showed superior performance in root extractions compared to ethanol, methanol, and water. Naringenin yields were consistently 8–25 times higher from aerial parts and 9 times higher from roots with NADES, confirming their overall superiority. Although coumaric acid was poorly extracted, ferulic acid, quercetin, and to a lesser extent rosmarinic acid, were significantly more concentrated in NADES extracts, particularly those using glucose- and glycerol-based mixtures.

The extraction efficiency varied depending on the solvent used and the plant part analyzed. For the aerial parts, the NADES composed of glycerol and citric acid demonstrated higher efficiency than 70% ethanol, methanol, and water in extracting gallic, chlorogenic, ferulic acids, epicatechin, quercetin, and naringenin. For caffeic acid, this NADES was more effective than methanol and water, and equally effective as 70% ethanol. However, ethanol and methanol were more efficient in extracting para-hydroxybenzoic acid. None of the solvents yielded significant quantities of rosmarinic or coumaric acid from the aerial parts.

For the roots, the glycerol/citric acid NADES was more effective in extracting gallic, chlorogenic, caffeic, and ferulic acids, as well as epicatechin, quercetin, and naringenin. However, para-hydroxybenzoic, coumaric, and rosmarinic acids were either present in trace amounts or not detected. The efficiency of glycerol/citric acid NADES is supported by previous research demonstrating its capacity to extract high levels of phenolic compounds from olive leaves, with extracts showing high total phenolic content [26]. Similar results were found in a study where citric acid-based NADES proved highly efficient for extracting phenolic compounds from Araza (*Eugenia stipitata*), a fruit native to the Amazon rainforest, using UAE [27].

The glycerol/urea NADES showed superior performance in extracting gallic, para-hydroxybenzoic, caffeic, and ferulic acids, as well as epicatechin and naringenin from chicory aerial parts compared to standard solvents. For chlorogenic acid, this NADES was only more effective than water, while coumaric and rosmarinic acids and quercetin were not extracted in significant quantities. From the roots, glycerol/urea extracted higher levels of gallic, caffeic, ferulic acids, epicatechin, naringenin, and quercetin than conventional solvents, whereas chlorogenic, para-hydroxybenzoic, and rosmarinic acids were not efficiently extracted, and coumaric acid was undetected. A 2020 study confirmed the efficacy of glycerol-based NADES in extracting phenolic compounds from processed olive oil, often surpassing conventional solvents [28].

The glucose/urea NADES enabled higher yields of gallic, chlorogenic, para-hydroxybenzoic, caffeic, ferulic acids, epicatechin, and naringenin from the aerial parts of chicory, outperforming 70% ethanol, methanol, and water. Coumaric and rosmarinic acids and quercetin were not significantly extracted by any solvent. From the roots, this NADES enabled superior extraction of gallic, caffeic, and ferulic acids, epicatechin, quercetin, and naringenin. These findings align with a study showing that onion peel extracts obtained using urea-based NADES demonstrated higher antioxidant activity compared to alcoholic extracts [29].

The glucose/lactic acid NADES exhibited high extraction efficiency for gallic, chlorogenic, coumaric, and ferulic acids, as well as epicatechin, quercetin, and

naringenin from the aerial parts of chicory. Its efficiency for caffeic acid was comparable to 70% ethanol, while para-hydroxybenzoic and rosmarinic acids were not significantly extracted. For root extractions, this NADES yielded higher concentrations of gallic, chlorogenic, caffeic, ferulic acids, epicatechin, quercetin, and naringenin, with minimal levels of para-hydroxybenzoic, coumaric, and rosmarinic acids. A previous study confirmed the superior performance of glucose/lactic acid NADES among ten tested systems in extracting phenolic compounds from cherries [30]. Similar outcomes were reported in a study demonstrating that lactic acid-based rosemary extracts exhibited greater antioxidant capacity than ethanol-based extracts [31].

The glycerol/glucose NADES proved to be an exceptionally effective extractant for all bioactive compounds from the aerial parts of chicory, with particularly high yields of para-hydroxybenzoic and caffeic acids. It also demonstrated better performance in root extractions, where all target compounds, except for coumaric acid, were present in higher concentrations than in standard solvent extracts. One study found that glycerol-based NADES extracts of flavonoids and phenolic acids from Plantago major exhibited higher antibacterial activity and lower genotoxicity than ethanol extracts [32].

The observed differences in extraction efficiency among the tested NADES systems can be attributed to their distinct physicochemical properties. The type of HBD and HBA determines the polarity, viscosity, and hydrogen bonding capacity of each NADES, which directly affect the solubilization of phenolic compounds. For example, choline chloride-based NADES containing organic acids showed higher efficiency in extracting phenolic acids such as gallic and caffeic acid, likely due to favorable hydrogen bonding interactions. In contrast, sugar- and polyol-based NADES exhibited lower extraction yields, possibly as a result of higher viscosity, which reduces diffusivity and mass transfer. Additionally, the water content of the NADES plays a crucial role in modulating solvent polarity and lowering viscosity, thereby improving extraction performance. These factors together explain the variability in extraction efficiency among different NADES formulations.

Despite some limitations in extracting certain minor compounds like rosmarinic acid, the overall results strongly support the use of NADES as efficient, eco-friendly alternatives to traditional organic solvents. Their superior selectivity, biodegradability, and low toxicity make them promising candidates for applications in food, pharmaceutical, and cosmetic industries aiming to incorporate green extraction technologies.

4 Conclusion

The evaluated NADES systems demonstrated superior extraction efficiency compared to conventional solvents in chicory root and aerial parts. These results highlight the potential of NADES in phenolic extraction, although further studies on other plant matrices and solvent compositions are required to confirm the broader applicability of this approach.

By leveraging an innovative, sustainable approach combining UAE with tailored NADES formulations, this work contributes to the growing body of evidence supporting the application of green chemistry in bioactive compound extraction. The findings provide a strong basis for further development of eco-conscious extraction protocols.

Importantly, the tested NADES in this study often outperformed traditional solvents, while avoiding their environmental and toxicological drawbacks. This underscores the promise of NADES as sustainable alternatives in natural product extraction. Nevertheless, broader validation across diverse systems is required before general conclusions can be drawn.

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