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Acmella oleracea Metabolite Extraction Using Natural Deep Eutectic Solvents

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Abstract: For plant metabolite extraction, natural deep eutectic solvents (NADESs) have many benefits over conventional solvents and ionic liquids. These advantages include high solubility and extraction ability, a low melting point (<100 °C), low toxicity, environmental friendliness, recyclability, and better biodegradability. This study analyses a natural deep eutectic solvent for *Acmella oleracea* (*A. oleracea*) metabolite extraction, considering the following process parameters: temperature, component ratio in the eutectic solvent, water addition, solid/liquid ratio, and extraction duration. NADESs were synthesised using a simple heating method, and the synthesis of the NADESs was verified by Fourier transform infrared spectroscopy (FTIR). In terms of total polyphenol content (TPC) and flavonoid content (TFC), the betaine/propanediol ratio in a NADES of 1:3 and S/L = 1:5 yielded the highest efficiency. A value of 8.37 mg GAE/mL was obtained for TPC by ultrasound-assisted extraction with 40% water addition, 25 °C extraction temperature, and 60 min contact time. The best result in terms of TFC was 14.50 mg QE/mL obtained through ultrasound-assisted extraction with 0% water added, 25 °C extraction temperature, and 60 min contact time.

Keywords: *Acmella oleracea*; liquid–solid extraction; eutectic solvent; NADES

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1. Introduction

Medicinal plants are a valuable source of chemicals with therapeutic activity that can be used as models to create new pharmaceutical or dermatological plant-derived formulations. *A. oleracea* is a flowering herb belonging to the *Asteraceae* family and is used as a medicinal remedy in various parts of the world due to its high content of multiple bioactive compounds: N-alkylamides, phytosterols, tannins, steroid glycosides, alkaloids, and phenolic compounds [1–3]. The Flavour and Extract Manufacturers Association (FEMA, in 2000) and the European Food Safety Authority (EFSA, in 2015) have both rated *A. oleracea* as safe (GRAS #3783) due to its low toxicity [1]. The bioactive substances found in *A. oleracea* have been shown in the literature to have extraordinary pharmacological properties: anti-inflammatory, diuretic, antipyretic, antifungal, antiplasmodial, local anaesthetic, and antioxidant effects [4]. At present, it is used in a number of industries, including dentistry, nutraceuticals, cosmetics, medicines, and food and drinks. The antioxidant and anti-inflammatory properties of *A. oleracea*, as well as its possible role in reducing the risk of oxidative stress-related disorders, have been reviewed by Rahim et al. (2021), proving its health beneficial effects [4].

The effectiveness of bioactive chemicals is contingent upon various aspects, one of which is their isolation from the plant matrix. The first stage in eliminating undesirable materials and isolating phytochemical compounds—which are mostly bioactive natural

products utilised as sources for the production of therapeutic ingredients—is phytochemical extraction. The choice of solvent is an important consideration in these processes, particularly in light of the growing restrictions on common solvents due to regulatory pressures regarding the detrimental effects of volatile organic compounds (VOCs) and their flammability, high toxicity, and non-biodegradability. Traditional solvents are excellent at removing bioactive compounds and are widely used to prepare extracts from natural resources. However, because of their inherent toxicity, volatility, thermal instability, solubility, and poor selectivity, they present significant drawbacks. The use of organic solvents, which are frequently hazardous and poisonous, in the extraction process results in their presence in the atmosphere as pollutants, even if the solvents are separated from the extracts before use. Furthermore, they persist in both the extracts and the raffinate, reducing their purity and necessitating further purification, which uses a lot of energy and renders conventional methods unfeasible. In addition to demands from consumers and other industries, from an environmental point of view, the elimination of volatile organic solvents is a necessity. Green solvents can be used to ensure that the extracts produced are free of hazardous organic solvent residues and do not require additional processing. This renders these methods commercially viable from an industrial perspective.

In order to create novel solvents with safer ecotoxicological profiles, more affordable prices, and advantageous qualities for various industrial and commercial operations, a great deal of research has been conducted [5,6]. It is critical that these solvents are renewable, biobased, biodegradable, minimally toxic, and efficient due to the analytical procedures involved in removing high-value chemicals from natural matrices. Green solvents are substitute solvents that satisfy "green" standards, meaning they are safe for the environment, and have emerged as a promising option in recent years.

A DES is a combination of a hydrogen-bond donor (HBD), which can be an alcohol, carboxylic acid, amine, or polysaccharide, and a hydrogen-bond acceptor (HBA). Betaine, obtained from renewable resources as a by-product of sugar production, has recently been utilised in the DES synthesis process, as it is less hazardous (high biodegradability and low toxicity profile) and has a low price. Because the combination of an HBD and an HBA has a lower melting point than any of its constituent parts, it can be used as an extractive solvent. If DES components are of natural origin, various plant metabolites (sugars, alcohols, amino acids, organic acids) are used, the DES is referred to as an NADES, and it possesses the ability to transport compounds from the cells.

In this context, due to their many benefits, including safety, affordability, biodegradability, thermal and chemical stability, environmental friendliness, and food grade, natural deep eutectic solvents (NADESs) could be viewed as viable candidates and alternatives to conventional ones. Moreover, due to NADESs' properties, the obtained extract does not require additional steps for isolation or purification; NADESs might serve as active components, and the extract could be employed directly in medicinal or cosmetic formulations [7–9].

Because of their abundance, affordability, recyclable nature, and appeal to the food, cosmetic, and pharmaceutical industries, they have demonstrated the most promise in the field of green chemistry [10–12]. In order to obtain high-quality extracts from a variety of plants, including native Greek medicinal plants [10], olive pomace [13,14], lemon verbena [15], peppermint and lemon balms [16], sour cherry pomace [17], wild thyme (*Thymus serpyllum* L.) [18], and blueberries [19], numerous studies have so far successfully analysed NADES extraction. Potential biological activity, bioavailability, and the ability to prepare NADESs in an infinite number of solvent combinations are all important advantages of their use [19,20]. In the cosmetic field, due to their special qualities, they can be suggested for a variety of pharmaceutical and cosmetic uses, from the creation of biocompatible drug delivery response systems to sustainable extraction and the acquisition of ready-to-use chemicals [21]. Thus, the regulatory framework and ecological chemistry requirements that cosmetic active ingredients must comply with have been established, so that in time they support innovation in the cosmetic industry [22].

Studies conducted by Rashid et al. (2023) showed that for flavonoid and polyphenol extraction from apple pomace, NADES provided higher yield than 70% ethanol [23]. Meng et al. (2018) obtained the following recovery rates for four bioactive flavonoids: 86.87% for quercetin, 98.34% for naringenin, 97.64% for kaempferol, and 98.89% for isorhamnetin from *Pollen typhae* using a NADES consisting of choline chloride and 1,2-propanediol [24]. This extraction efficiency was significantly higher than that reported when using traditional solvents like methanol, ethanol, or water. Because they are more efficient at extracting polyphenols than typical solvents and are also more ecologically friendly, NADESs are the better option for polyphenol extraction. These solvents demonstrate their versatility by allowing for the extraction of a broad variety of polyphenols, such as anthocyanins, flavonoids, and hydroxycinnamic acids [18]. NADESs are a viable and efficient substitute for traditional organic solvents because they provide softer extraction conditions and enhanced antioxidant compound extraction efficiency as well as antioxidant activity. The inability to efficiently separate the extracted bioactive compounds from NADESs in order to obtain dry extracts is a drawback that is frequently discussed in the literature regarding the extractions employing NADESs. This disadvantage can, however, be turned into a benefit because NADESs enhance the final product, and the best possible extract has been effectively used in the production of a cosmetic cream [21,22].

The majority of published articles on *A. oleracea* metabolites are focused on extraction through conventional methods [25–28]. Bellumori (2022) examined the amount of total phenols in the hydroalcoholic and hexane extracts of the aerial parts and roots of *A. oleracea* under three distinct extraction settings: 10 min of sonication at 60 °C, 50 min of magnetic stirring followed by 10 min of room-temperature sonication, and 10 min of room-temperature sonication. The findings demonstrated that, in comparison to the samples extracted using the other two methods, the hydroalcoholic extract of the roots and aerial parts extracted under the first conditions (10 min of sonication at 60 °C) exhibited a significantly higher content of phenolic compounds; the phenolic compounds in the roots nearly doubled in quantity and reached approximately 3.5 mg/g dry matter in the aerial parts [29]. Abeysinghe (2014) used 80% methanol for polyphenol extraction from *A. oleracea* using a combined extraction technique with vortexed samples for 15 min and maceration at 6 °C for 40 min, obtaining 10.99 mg GAE/g dry weight of total polyphenol content [30].

The studies on metabolite extraction from *A. oleracea* have focused on the extraction of spilanthol (the main N-alkylamide from *A. oleracea*) with anti-inflammatory effects or the production of extracts with insecticidal or acaricidal activity. The main solvents used were ethanol, methanol, hexane, and supercritical CO_2. Besides spilanthol, which is the most abundant N-alkylamide found in *A. oleracea*, high quantities of polyphenols—vanillic acid, trans-ferulic and trans-isoferulic acid, and scopoletin—are also present. The extraction of these compounds using an appropriate solvent could lead to the obtainment of an extract with potential for direct application. NADESs can be considered safe solvents, and their capacity to stabilise throughout the extraction of phenolic compounds suggests that they can be used to produce plant extracts that are suitable for human consumption [31]. Betaine-based NADESs were used in the following fields of extraction: the extraction of protein from calf blood, nutraceutical compounds from coffee grounds, and the selective extraction of nitrogen from gasoline [32–34]. Fuad and Nazir examined the physicochemical characteristics of eleven betaine-based NADESs, including one containing 1,2-propanediol, and evaluated their viscosity, density, polarity, pH, surface tension, electrical conductivity, water addition effects, and thermal stability [35].

The current study's objective was to analyse the extraction process of *A. oleracea* using a biobased NADES as an alternative to traditional solvents, keeping in mind the growing concern over environmental effects. The influence of the following process parameters was analysed for classical extraction and ultrasound-assisted extraction: temperature, component ratio in the eutectic solvent, water content in the NADES, liquid/solid ratio, and extraction duration. To the best of our knowledge, no research has been conducted on the extraction of polyphenols and flavonoids from *A. oleracea* using eutectic solvents, despite

numerous reports of studies on the NADES extraction of various bioactive compounds such as anthocyanins, polyphenols, flavonoids, and catechins. As the use of NADESs can stabilise bioactive compounds, enhance their biological activity, and improve their bioavailability, the obtained extract could be used in cosmetic product formulation, as both components of NADESs are admitted in cosmetic products [21], and the antioxidant activity is the result of the synergism between the components of the polyphenol mixture. This helps in creating a production line with the fewest possible processing steps and producing an extract that is secure, non-denatured, and biodegradable.

2. Materials and Methods

2.1. Materials

The used plant was *A. oleracea* (Figure 1) from a culture that was acclimatised in Romania by a member of our team. This culture was located in Popricani, Iași County, Romania. After harvesting the plant, the aerial parts were separated from the roots and dried for six to seven weeks in a cool, shaded place. The dried plant was then stored in brown glass containers to prevent the absorption of atmospheric moisture and the oxidising action of ultraviolet rays. At the moment of use, the plant was ground with a centrifugal mill and sieved to obtain particles with a size of 1–3 mm.

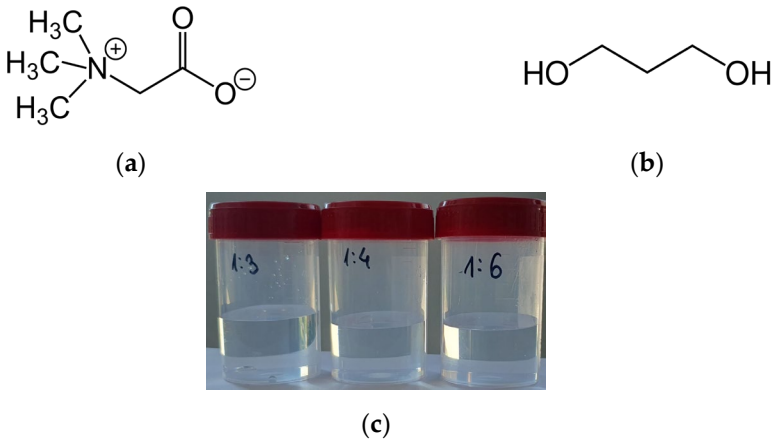
Figure 1. From *A. oleracea* plant to *A. oleracea* powder.

For the extraction, NADESs based on betaine (trimethyl glycine) as a hydrogen-bond acceptor (HBA) and propanediol (propane-1,3-diol) as a hydrogen-bond donor (HBD) at molar ratios of 1:3; 1:4, and 1:6 were synthesised. These two components selected to prepare the NADES mixtures were selected after consulting the official documents that approve and certify the quality of (dermato)cosmetic and pharmaceutical products (Regulation on cosmetic products: Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009; CIR (Cosmetic Ingredient Review) regulations and data from the COSMILE database). Also, their properties mean that the extract in a NADES can be fully considered a true asset for cosmetic products, without any intervention being necessary on it, in the form of a "ready-to-use ingredient" [21]. Studies have shown that the ingredient poses no risk of irritation or toxicity when commonly used in personal care products.

2.2. Methods

2.2.1. Preparation of NADESs

Obtaining a clear NADES mixture (Figure 2) without traces of crystallisation was performed by repeated heating and cooling: (i) The mixture was first heated at a temperature of 80 °C with constant stirring until the formation of a clear liquid, followed by cooling at 25 °C; (ii) the obtained mixture was reheated to a temperature of 80 °C, with intermittent mixing, and allowed to cool to an ambient temperature of 25 °C. These successive heating and cooling operations have the role of ensuring a good solubilisation of the betaine to obtain a homogeneous mixture without traces of crystallisation.



(**a**) (**b**)

(**c**)

Figure 2. Chemical structure of NADES components: (**a**) betaine and (**b**) propane-1,3-diol. Prepared NADES solutions (**c**).

2.2.2. Physicochemical Characterisation of NADESs

The density was calculated by measuring the mass of 1 mL of the mixture using a KERN ADB 100-4 analytical balance (Balingen, Germany). The pH values of the samples were determined using a digital pH Meter (Hanna Instrument, Nusfalau, Romania). The electrical conductivities of the NADESs ware measured using a portable Hanna Instruments conductometer (Hanna Instruments, Jakarta, Indonesia). All analyses were performed at atmospheric pressure and at 25 ± 0.5 °C. Microscopic images were taken of samples of the solvents stored under normal temperature conditions using a Euromex BSope binocular microscope with a digital video camera and Eplan achromat objectives at a total magnification of 100×. The photos were taken using dark field and polarisation (Euromex, Arnhem, The Netherlands).

2.2.3. Fourier Transform Infrared Spectroscopy Characterisation of NADESs before and after Extraction

Fourier transform infrared (FTIR) spectroscopy was used to characterise the successfully synthesised NADESs. The spectra of the NADESs were recorded by an FTIR Thermo Nicolet IS50 (Madison, WI, USA) spectrometer equipped with ATR and ESP modules in real-time mode with a resolution of 4 cm^{-1} and an incident angle of 45° over 30 s. The FTIR spectra were collected from 4000–600 cm^{-1} at room temperature.

2.2.4. Extraction Methods

The used extraction methods were as follows: *maceration* at room temperature (25 °C) with vibrational mixing at 1200 rpm (M) and *ultrasound-assisted extraction* (sonoextraction—US) to obtain vegetal extracts from the *A. oleracea* plant with the highest content of active compounds (Figure 3). Variables studied for their effects on the extraction process using NADESs included the S/L ratio (1:5; 1:15, and 1:25), temperature (25 °C, 45 °C, and 60 °C), composition of extraction solvent (betaine: propanediol = 1:3; 1:4, and 1:6), and extraction time (15 min, 30 min, 45 min, and 60 min).

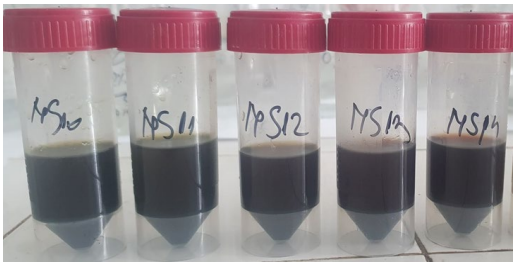


Figure 3. Vegetal extracts from *A. oleracea* plant in NADES solvents.

2.2.5. Determination of Total Polyphenol Content (TPC) and Flavonoids (TFC)

The Folin–Ciocalteu technique [36] was used to determine the total polyphenol content (TPC). The findings were expressed as mg of gallic acid equivalent (GAE) per mL extract (mg GAE/mL extract), taking sample dilution into consideration. Two sets of analyses were performed. The standard calibration curve was produced for a range of gallic acid concentrations using the same conventional techniques.

A spectrophotometric-based method was used to measure the quantity of flavonoids (TFC) in a 2% $AlCl_3$ solution that was prepared in methanol [37]. The findings were represented as mg of quercetin equivalent (QE) per millilitre of extract (mg QE/mL).

All determinations were made in duplicate.

3. Results

3.1. Characterisation of NADESs

3.1.1. Physical Characterisation of NADES Extraction Solvents

The formation of a eutectic mixture—a combination of two primary components, namely an HBD and an HBA at a predefined molar ratio—with a melting point lower than that of either of the components was the basis for the expression of NADESs. The values of physical properties measured for the NADESs used for solid–liquid extraction are presented in Table 1. The obtained results are similar to reported data in the literature [38]. A microscope slide with a drop of NADES on it was examined under a polarised light-field microscope, obtaining the images presented in Table 1.

Table 1. Physical properties of the used NADESs.

NADESs	Physicochemical Properties			
	pH	Conductivity	Density (g/mL)	Optic Images
NADES 1 (1:3 = betaine/propanediol)	9.08	0.00	1.146	
NADES 2 (1:4 = betaine/propanediol)	8.81	0.00	1.141	
NADES 3 (1:6 = betaine7propanediol)	8.57	0.00	1.087	

3.1.2. FTIR Characterisation

The formation of a eutectic solvent is characterised by measuring the hydrogen bonding between the HBA and the HBD. By examining the infrared spectra of the precursor materials and the final eutectic combination, the evidence of NADES synthesis was obtained. Figure 4 shows the FTIR spectra of betaine, propanediol, and their corresponding eutectic mixture. The preservation of their characteristic bands, which reflect the functional groups involved in the interactions among NADES components, was observed and confirm the eutectic solvent formed.

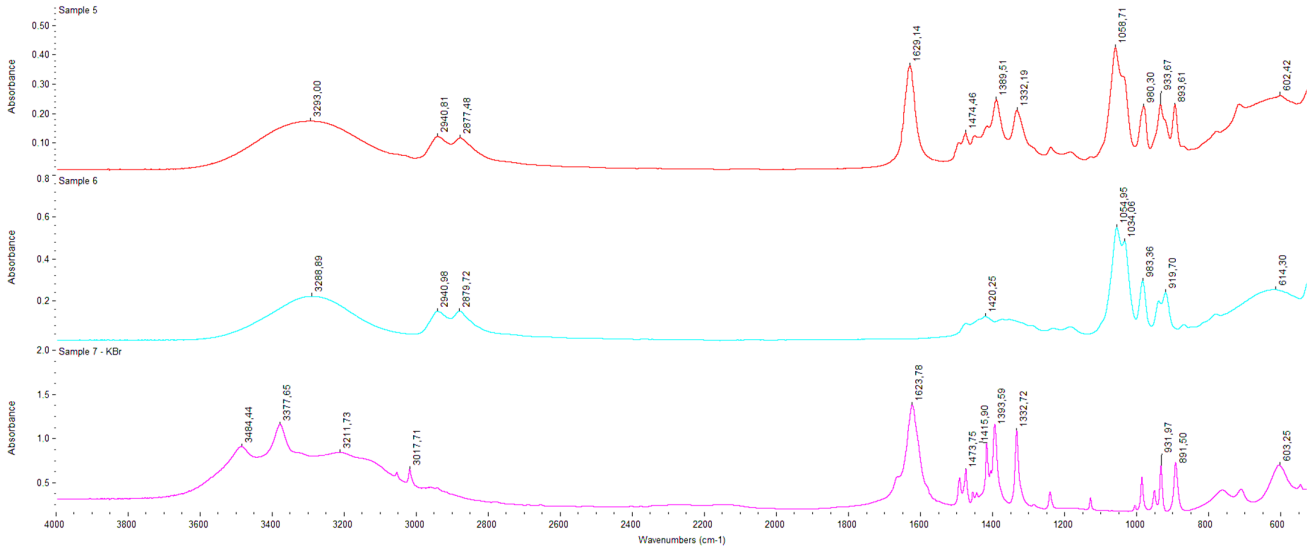
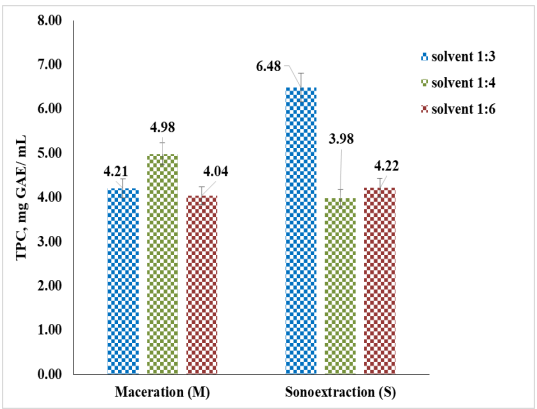


Figure 4. FTIR spectra for synthesised NADESs: 5: betaine/propanediol mixture (1:3 molar); 6: propanediol; 7: betaine.

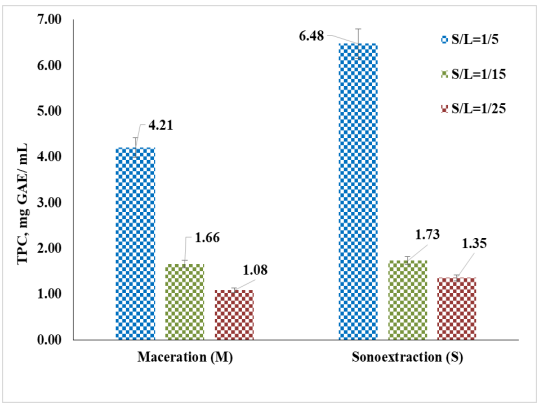
3.2. Total Polyphenol Content (TPC) and Flavonoids (TFC)

The samples from the extracts obtained under different conditions by the two extraction methods selected were filtered and centrifuged, and then the total contents of polyphenols and flavonoids were quantitatively determined. The results were expressed for flavonoids as mg of quercetin equivalent (QE) per mL (mg QE/mL) and for polyphenols as mg of gallic acid equivalent (GAE) per mL (mg GAE/mL). The findings of these experiments, which were conducted in duplicate, are shown in Figures 5a–e and 6a–e, depending on the extraction method and operating conditions.

For a better analysis of NADES performance, the extraction yield was evaluated and compared with conventional extractions. The results regarding the contents of polyphenols and flavonoids of *A. oleracea* extracts obtained after extraction with propanediol [39] and NADES are presented in Table 2.

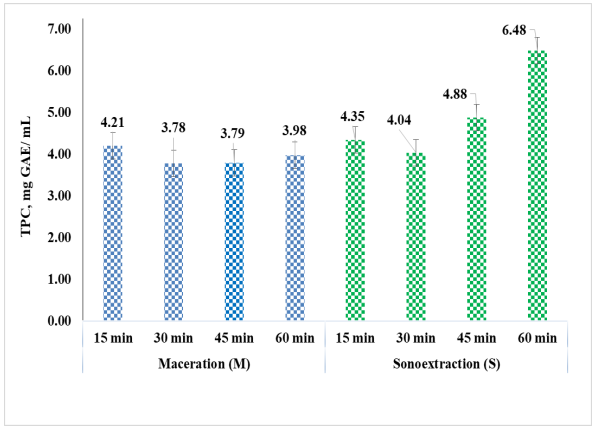


(**a**)



(**b**)

Figure 5. *Cont.*



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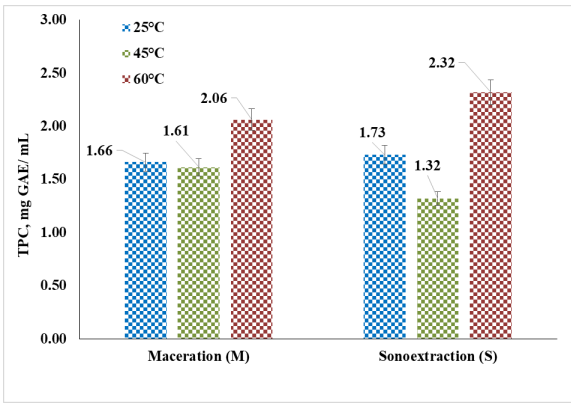

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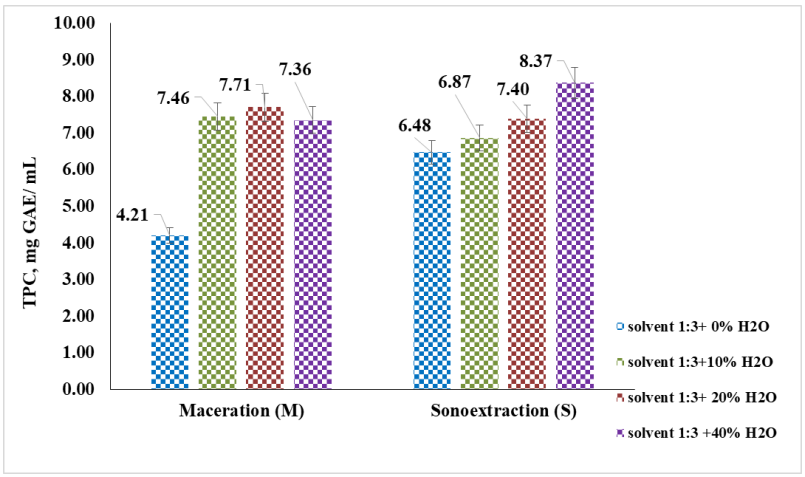
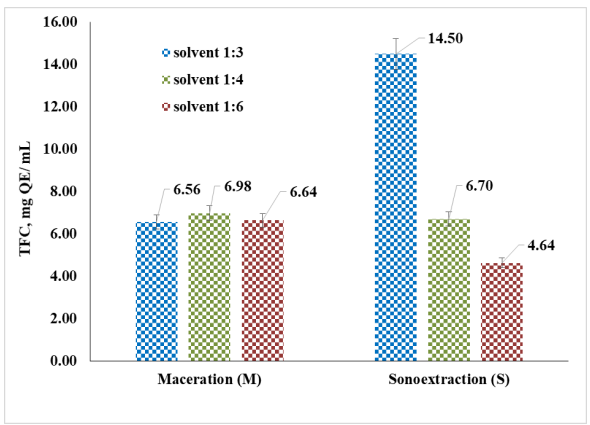
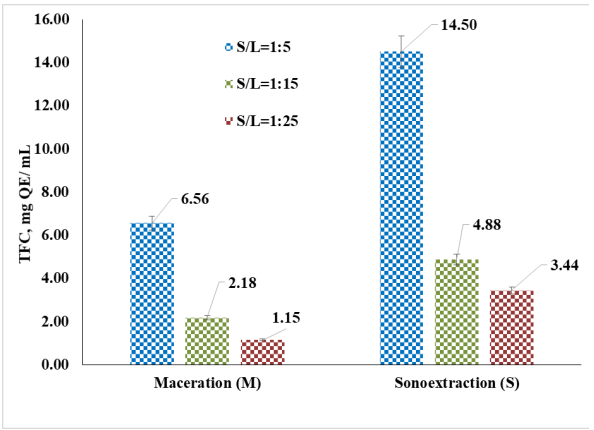


(**e**)

Figure 5. Total polyphenol content (TPC) in mg GAE/g determined comparatively for extraction methods used depending on physical parameters considered. Conditions: (**a**) S/L = 1:5, 25 °C extraction temperature, M = 15 min, S = 60 min; (**b**) solvent composition = 1:3, 25 °C extraction temperature, M = 15 min, US = 60 min; (**c**) solvent composition = 1:3, S/L = 1:5, 25 °C extraction temperature; (**d**) solvent composition = 1:3, S/L = 1:15, M = 15 min, US = 60 min; (**e**) S/L = 1:5, US = 60 min and M = 15 min, 25 °C extraction temperature.



(**a**)



(**b**)

Figure 6. *Cont.*

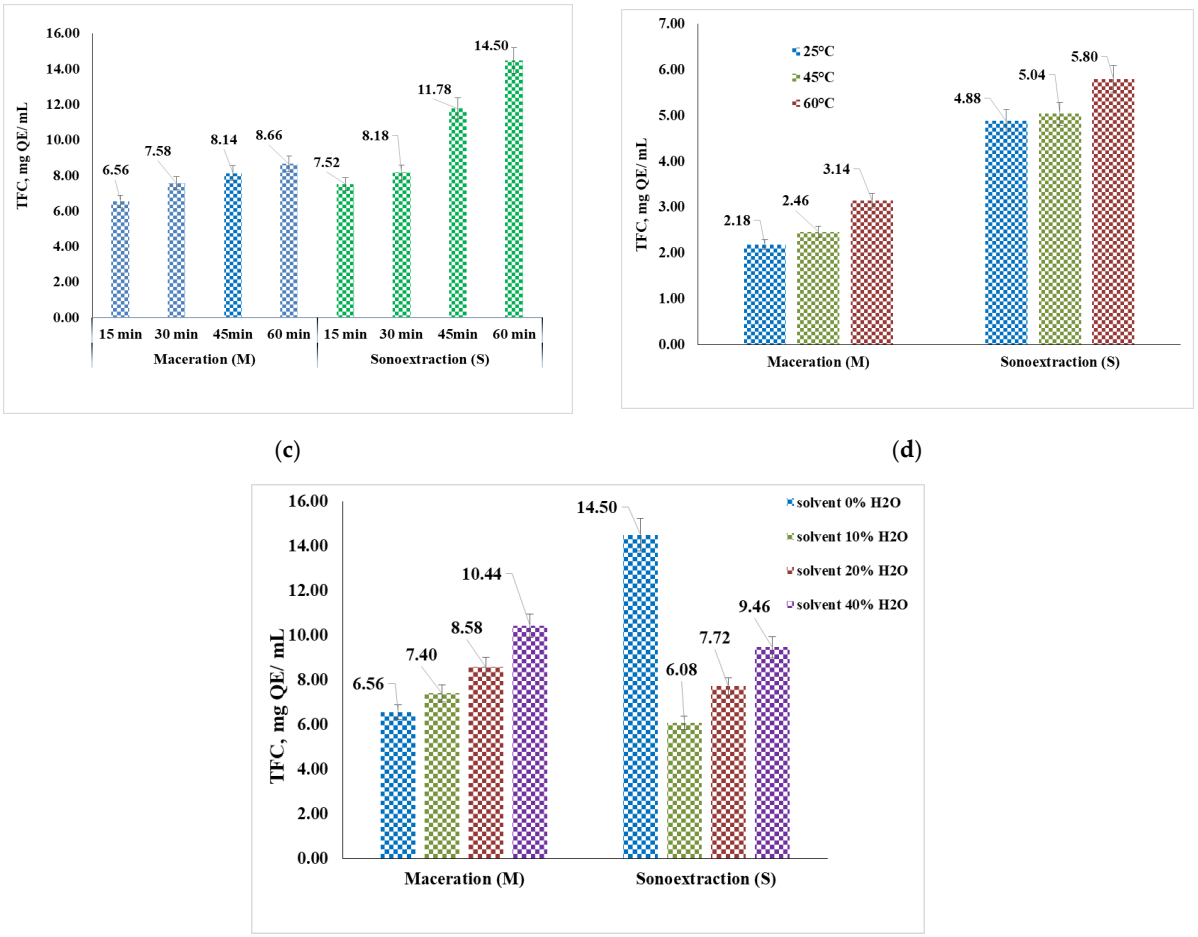


Figure 6. Flavonoid content (FC) in mg QE/g determined comparatively for extraction methods used depending on physical parameters considered. Conditions: (**a**) S/L = 1:5, 25 °C extraction temperature, M = 15 min, S = 60 min; (**b**) solvent composition = 1:3, 25 °C extraction temperature, M = 15 min, US = 60 min; (**c**) solvent composition = 1:3, S/L = 1:5, 25 °C extraction temperature; (**d**) solvent composition = 1:3, S/L = 1:15, M = 15 min, US = 60 min; (**e**) S/L = 1:5, US = 60 min and M = 15 min; 25 °C extraction temperature.

Table 2. The content of polyphenols and flavonoids in extracts based on propanediol and NADES.

Extracted Compound	Type of Solvent/Method Characteristics	
	Propanediol	**NADES (Betaine/Propanediol = 1:3)**
Polyphenols, mg GAE/g	3.39 (M: 60% propanediol concentration, 7 days, S/L = 1:5) 3.00 (M: 40% propanediol concentration, 30 days, S/L = 1:5)	6.48 (S: 60 min, S/L = 1:5, 25 °C) 4.21 (M: 15 min, S/L = 1:5, 25 °C)
Flavonoids, mg QE/g	8.98 (M: 40% propanediol concentration, 30 days, S/L = 1:15) 8.37 (M: 60% propanediol concentration, 14 days, S/L = 1:20)	14.5 (S: 60 min, S/L = 1:5, 25 °C) 8.66 (M: 45 min, S/L = 1:5, 25 °C)

4. Discussion

4.1. Physical Characterisation of NADES Extraction Solvents

The values measured for the analysed eutectic mixture properties (Table 1) demonstrate a clear (transparent liquid), homogenous solvent that maintained its stability without re-crystallisation during storage for all three combinations of HBA/HBD. This behaviour shows a stable molecular structure with balanced intermolecular forces between the two NADES components through hydrogen bonding interactions. The uniform black colour

in the microscope images (Table 1), without whitish "spots", suggests the formation of a homogeneous solution without crystallisation centres. It is thus obvious that the eutectic mixtures were formed at certain eutectic compositions, establishing a balanced intermolecular force between the HBA and the HBD through hydrogen bonding interactions and resulting in a stable molecular structure within the eutectic system, therefore preventing any formation of crystals. The information obtained is in accord with what Fuad and Nadzir also presented in their study regarding the obtainment and characterisation of eutectic mixtures, including those based on betaine [35].

4.2. FTIR Characterisation

The analysis of FTIR spectra from Figure 4 confirms the formation of a eutectic mixture between betaine and propanediol (1:3) by preserving the characteristic bands of the individual compounds, which reflect functional groups involved in the interactions between NADES components as well as the hydrogen bonds formed between the components of the mixture. Betaine showed an absorption band at 1623 cm^{-1} specific to the carbonyl (C=O) group, while propanediol presented bands at 3288 cm^{-1}, attributed to the hydroxyl (O-H) groups in the molecules. In Figure 4, an intense absorption band between 3000 and 3500 cm^{-1} can be observed for the NADES spectrum corresponding to the stretching of O-H groups in the molecules, as well as an absorption peak specific to C=O at 1629 cm^{-1}, in good agreement with betaine-based NADES spectra available in the literature. The NADES spectra recorded a shift of the C=O peak from 1623 to 1629 cm^{-1} due to an increase in the electron density of the oxygen from the carbonyl functional group, thus proving hydrogen bond formation in the NADESs [18].

4.3. Determination of Total Polyphenol Content (TPC) and Flavonoids (TFC)

Solubility is an extremely important aspect of polyphenol extraction from vegetable matrices to ensure that the process successfully maximises the yield of plant metabolites. By promoting or inhibiting specific molecular interactions, solvents can affect a solute's solubility and stability [18,40]. The HBA and HBD components of NADESs cause differences in polarity, viscosity, and dissolving ability, influencing the extraction efficiency. Several studies concluded that the NADES extraction of plant metabolites resulted in better yields of bioactive compounds, when compared to those for traditional solvents, due to their higher solubility.

Due to their similar polarities and chemical interactions—such as van der Waals forces, H-bonds, and dipole–dipole interactions—polyphenols dissolve into NADES during the extraction process, which also involves mass transfer from a solid to a liquid phase, facilitated by sonoextraction (US). US is a technique that improves the interaction between the active ingredients in the vegetable matrix and the solvent for an increased extraction efficiency in a short time. It works by causing cell wall tissue to rupture due to cavitation effects. NADESs have high extraction efficiencies because of their high hydrogen bond basicity. The diffusion of polyphenol molecules outside of plant cells is facilitated by the high solubility of polyphenols and the diffusivity of NADES. The H-bond interactions between the NADES and polyphenol are the most important driving force for the extraction, and they dominate the other polyphenol–polyphenol electrostatic forces. This makes it more capable of penetrating the cell wall's composition and promoting successful molecular interactions between the NADES and plant cellulose chains. Moreover, NADESs can donate and receive protons and electrons, which enables them to create hydrogen bonds with phenolic compounds to increase their solubility.

In the case of polyphenol content in the NADES extracts presented in Figure 5, the following parameters have been investigated: molar ratio between the HBA and HBD forming the solvent; solid/liquid ratio, extraction duration, temperature, and the amount of water added to each NADES. Considering the three NADESs obtained using different betaine/propanediol ratios, it can be observed that the composition mixture of 1:3 shows the best results (6.48 mg GAE/g), with the influence in the case of maceration being less

important compared to that of sonoextraction. The particular NADES with a 1:3 HBA-to-HBD ratio was selected as the best extraction solvent for further experiments (Figure 5a).

Analysing the S/L ratio's (Figure 5b) influence on the polyphenol content, the best results were recorded in the case of sonoextraction (6.48 mg GAE/mL), followed by maceration (4.21 mg GAE/mL) in the case of the 1:5 ratio, with a higher solvent volume determining the extracted polyphenols' dilution. This is by far the most efficient solid/liquid ratio, assuring the necessary contact area between the solid matrix and NADES, thereby favouring a higher polyphenol yield. The increase in polyphenol concentration obtained by decreasing the S/L ratio is an advantage, as less NADES is needed, and also, less energy is required for mixing.

Taking into account the extraction time (Figure 5c), under the selected conditions, the best values of the polyphenol content were obtained in the case of maceration for 60 min and S/L = 1:5 (6.48 mg GAE/mL). It can be observed that with the increase in the extraction time, in the case of both methods, an increase in the amount of extracted polyphenols is also observed.

The temperature (Figure 5d) also has an influence on the quantity of polyphenols extracted by changing the viscosity of the solvent and increasing the permeability of the cell membrane of the plant. The best results were obtained in the case of sonoextraction at 60 °C (2.32 mg GAE/mL).

Figure 5e shows the results in the case of using the NADES 1:3 mixture as an extraction solvent in combination with different percentages of water, added mainly to reduce the NADES's relatively high viscosity. It is observed that in the case of maceration, the influence between the three ratios is insignificant. A greater difference is recorded between the simple NADES mixture and the one mixed with water: the addition of water almost doubled the amount of extracted polyphenols, its role being to permeabilise the cell membrane, facilitating the diffusion of the compounds and the extraction agent.

Flavonoids (anthocyanidins, chalcones, flavanones, flavones, isoflavones, and flavonols) are a group of naturally occurring phenolic chemicals that are produced by plants as bioactive secondary metabolites. Flavonoids provide plants their flavour, colour, and pharmacological properties (effective antioxidants—through the chelation of transition metal ions, flavonoids both bond free radicals like reactive oxygen species and stop their generation). In the case of the flavonoid content in the NADES extract, presented in Figure 6, the experimental results show an optimum betaine/propanediol ratio of 1:3 in this case as well, with a maximum flavonoid content of 14.5 mg QE/g, being more important in the case of ultrasound-assisted extraction (Figure 6a).

The study of the content of flavonoids in the extracts was performed according to the same criteria as in the case of polyphenols. They showed the following:

- Depending on the S/L ratio (Figure 6b), it can be seen that the best results were recorded in the case of sonoextraction (7.52 mg QE/g), followed by maceration (6.56 mg QE/g), using the 1:5 ratio. This result is in accordance with data from the literature, as sonoextraction usually provides a high extraction yield with the use of less solvent and energy compared to maceration.
- Considering the extraction time (Figure 6c), under the selected conditions, the best values of the flavonoid content were obtained in the case of sonoextraction for 60 min and S/L = 1:5 (14.5 mg QE/g). It can be observed that with the increase in the extraction time, in the case of both methods, an increase in the amount of extracted flavonoids is also observed.
- Depending on the temperature (Figure 6d), it can be seen that better results were obtained in the case of sonoextraction at 60 °C (5.8 mg QE/g). The temperature also influences the amount of extracted flavonoids through the action of changing the viscosity of the solvent and increasing the permeability of the cell membrane of the plant, and a high temperature also promotes the formation of more cavitation bubbles, which expand the solid–solvent contact area and diffusion.

- If we also take into account the composition of the solvent, pure or mixed with water in different percentages (Figure 6e), we can consider the results in the case of using the NADES 1:3 mixture as an extraction solvent in combination with different percentages of water. It is observed that in the case of maceration, the amount of flavonoids increases with the increase in the percentage of water in the extractant. In the case of sonoextraction, the largest quantity of extracted flavonoids is seen in the case of using the simple solvent without water. The percentage of water added influences the flavonoids extracted in a favourable way but without reaching the values obtained in the case of the solvent without water. As in the case of polyphenols, it can be said that the introduction of water into the extraction solvent has an important role depending on the extraction method used. Solvents can influence a solute's solubility and stability by facilitating or hindering particular molecular interactions. An ideal hydrophilicity may be reached with a particular volume of water; the extraction or solubilisation of flavonoids does not follow a solvent's increasing hydrophilicity in a proportionate manner, as certain phenolics require particular water levels for efficient extraction. This demonstrates that the type of NADES components at the proper mixing ratio and the water content in a NADES may be changed to alter the solvent's ability to dissolve target chemicals in plants.

Comparing the data on the content of polyphenols and flavonoids in *A. oleracea* extracts obtained from extraction with propanediol and NADES (Table 2), it was observed that the use of a NADES for the extraction of polyphenols and flavonoids from *A. oleracea* is more effective than the use of aqueous solutions of propanediol. Also, the most effective of the studied methods was sonoextraction, and under the conditions of 60 min, S/L = 1:5, and 25 °C, the highest values were obtained for both polyphenols and flavonoids. This investigation showed that *A. oleracea* may be a significant natural antioxidant source that could be used in delaying the onset of a number of disorders linked to oxidative stress.

The obtained results are comparable to the ones obtained by Bellumori et al. (2022), who obtained 3.5 mg/g phenolic compounds in *A. oleracea* aerial parts using a hydroalcoholic solution as a solvent and sonication for 10 min at 60 °C [29].

5. Conclusions

A. oleracea is a commonly used medical plant, particularly in the cosmetics industry, where its antioxidant-rich chemical components play a major role. Because of their exceptional solubilisation and physicochemical properties, NADESs have been deemed environmentally friendly solvents and are suggested for use in industrial applications. In this study, betaine was used as the HBA and 1,3-propanediol was used as the HBD in various molar ratios to successfully synthesise three NADESs. Their screening in the green extraction of polyphenols and flavonoids from *A. oleracea* showed that sonoextraction using NADESs is more efficient and less time-consuming than maceration in extracting polyphenols and flavonoids from *A. oleracea*. The best results for polyphenols were obtained after extraction with NADES 1:3 through sonoextraction (6.48 mg GAE/mL) under conditions of S/L = 1:5, 60 min, and 25 °C, and the largest amount of flavonoids was obtained after extraction with NADES through sonoextraction (14.5 mg QE/g) under conditions of S/L = 1:5, 60 min, and 25 °C.

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