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Development of Green Extraction Using Natural Deep Eutectic Solvent (NADES) for Separation Polyphenolic Compounds from *Spilanthes acmella*

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Abstract

Natural deep eutectic solvent (NADES) is an alternative to conventional solvents in polyphenol extraction. Its composition of choline chloride combined with fructose, choline chloride with lactic acid, choline chloride with glycerol, choline chloride with propylene glycol, and choline chloride with proline have been sucessfully set using the thermal mixing method. Choline chloride served as the hydrogen bond acceptor (HBA), while the others acted as hydrogen bond donors (HBDs) at a mole ratio of 1:2. This study aimed to extract polyphenols from the flowers, stalks, stems, and leaves of *Spilanthes acmella*, using NADESs. The extraction process was conducted by adding 20% water to the mixture with a ratio of the sample to solvent of 1:10 (w/v), for 60 min. The extract concentration was assessed through spectroscopic photometry using the Folin-Ciocalteu method. The result showed that the choline chloride-glycerol NADES was the most appropriate solvent for the extraction of polyphenols from the flowers of the plant. Meanwhile, the best NADES for the extraction from the stems, stalks, and leaves, was the mixture of choline chloride and fructose. The highest polyphenol concentration was obtained in the stems at 2545.192 mg/L. The yield obtained in the stems, leaves, stalks, and flower were 25.452, 6.284, 3.111 and 2.415 mg gallic acid equivalent/g (GAE/g), respectively. These findings suggest that NADES can be used as an eco-friendly alternative solvent for green extraction processes of active compounds from natural materials, especially polyphenols from *S. acmella*.

Keywords

extraction, polyphenols, NADES, *Spilanthes acmella*

1 Introduction

Plants are a potential source of bioactive compounds that are extensively utilized in various sectors, including industry, food, and healthcare. Active compounds including phenols, tannins, alkaloids, flavonoids, steroids, terpenoids, and glycosides extracted from the leaves, stems, seeds, fruits, and other parts of plants, are mostly used in the field of health, particularly for drug development [1, 2].

The solvent used in the extraction process needs to posses high selectivity, safety, neutrality, ease of separation from the target compound, low viscosity, a low boiling point, and economical viability. Typically, the extraction of active compounds from plants is performed using organic solvents. The commonly used types include hexane, ethyl acetate, chloroform, acetone, methanol, ethanol, and dichloromethane. The application of these compounds as solvents is attributed to the effective dissolving and extraction capabilities [1, 3, 4]. However, they are often used in large quantities, leading to high extraction costs. Organic solvents are relatively toxic, flammable, explosive, and difficult to decompose [5–7]. The usage as a solvent presents significant safety concerns, particularly in the extraction of materials used in food products. To overcome this, a safe and environmentally friendly alternative is needed.

The problems lead the developing a safe and environmentally friendly alternative called a green solvent. The development of green solvents started with the use of ionic liquid (IL) to replace organic solvents. This is a type of salt with specific physicochemical characteristics that can be modified through the combination of various

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cations and anions [8, 9]. IL has a lower melting point below 100 °C, and it consists of cations and anions combined through intermolecular interaction, such as ionic bonds, hydrogen bond, or van der Waals forces [9–11]. The salt has distinctive properties including low volatility, high thermal stability, and high solvation ability [12], prompting usage in various fields such as pharmaceuticals.

IL should not be present in food products because of their potential toxicity and composition of non-natural materials. The high solubility of IL in water is a significant threat to aquatic biota due to elevated stability, which hinders natural degradation [13, 14]. The toxicity of the salts arises from long-chain cation and anion components, and the level of toxicities is too high for conventional solvents in bacteria [13–15]. Some ILs also show harmful properties for humans and are highly toxic, hence, no longer recommended for usage [16].

As an alternative to IL, which is considered less environmentally friendly, a new solvent called deep eutectic solvent (DES) was adopted. It is a combination of several compounds with melting points that are lower than that of the constituent components. DES is prepared by combining salts of quaternary ammonium (acting as hydrogen bond acceptor, or HBA) with protic organic compounds such as urea, amide, or carboxylic acid (serving as hydrogen bond donor, or HBD), connected through hydrogen bonding [17]. The solvent is formed from the mixing and heating of several components consisting of HBA and HBD. In addition to being non-evaporative, non-inflammable, and less toxic. DES has low vapor pressure with high dissolving ability and thermal stability [18, 19].

DES is considered more sustainable and green than IL because the solution is prepared using simpler and faster procedures, requiring low heating. It is also environmentally friendly and easily degrading in water. Compared to IL, the solvent is cheaper to produce on industrial scale and safer [9, 19].

DESs have been developed and obtained from natural materials called NADES, natural deep eutectic solvent. NADES supports green technology in the green chemistry sector with the utilization of green solvents applicable in the food, pharmaceuticals, and cosmetics industries. Furthermore, it refers to a novel variation of DES, which is considered more "natural". This is because the eutectic compound component (HBD) is obtained from primary metabolites such as sugars, organic acids and bases [2, 20]. IL, DES, and NADES possess similar physicochemical characteristics such as low melting points and good dissolving ability for certain compounds. However, the use of NADES is wider and considered more environmentally friendly than conventional solvents. Some of its advantages include:

1. the components have simple structure and are available on the market in the form of bulk chemicals;
2. non-volatile during the extraction process and can be recycled;
3. low energy required for preparation;
4. low toxicity;
5. readily decomposing;
6. function similarly to organic solvents in extraction process;
7. remains stable even when exposed to elevated temperature;
8. and non-flammable [2].

The utilization of NADESs for dissolving bioactive compounds from natural materials have been extensively studied. Some examples of the bioactive compounds include pectin [21], curcuminoid [22], flavonoid extraction [23, 24], lignin [25], phenolic compounds [3, 23], and polyphenol [26]. The utilization of NADESs in extracting natural products have been limited by the extraction effectivity. Meanwhile, its traits as a green solvent show high potential to be utilized in active compound extraction from herbal plants. The solvent can be developed in pharmaceutical and cosmetic products.

Pharmaceutical products can be developed from herbal plants such as *Spilanthes acmella*. This plant is also called a medicinal plant because the roots, leaves, flowers, stems, and stalks can be used for health purposes. The main compound in *S. acmella* is spilanthol, occasionally adopted for the reduction of diseases associated with pain, such as tooth pain, and potentially stimulate salivary secretion [27, 28]. The features of spilanthol include antibacterial, anti-microbial, antioxidant, anti-inflammatory, and anesthetic effects [27–29].

The solvents used to extract spilanthol, are, e.g., methanol [30], ethanol, supercritical CO_2 [31, 32] or hexane [33]. Following extraction, the compounds are subjected to purification through TLC and/or HPLC methods [34]. Active compounds in *S. acmella* can also be extracted using the soxhletation method [35]. However, the utilization of organic compounds as solvents during the process of extraction is currently being avoided and replaced with more environmentally friendly solvents. The application of *S. acmella* extracts as herbal medicine should be conducted using a natural extraction process to prevent any negative effects. Therefore, green technology can be incorporated into the process of extracting active compounds. The utilization of NADES as a solvent, is considered safer in terms of the process, economical, and adoption in pharmaceutical products. Some active compounds in *S. acmella* include flavonoids, tannins, alkaloids, and phenol components (polyphenols), steroid glycosides, and terpenoids [35, 36]. Polyphenol compounds have many benefits, including antioxidant and anti-inflammatory effects. However, most extraction processes still use organic solvents. This study aimed to utilize NADESs with various component combinations for the extraction of polyphenol compounds from *S. acmella*. It is expected that the extract can be applied in the pharmaceutical, medical, and food sectors.

2 Materials and instruments

The materials of *S. acmella* was collected from Bogor, the region of West Java, located in Indonesia. The reagents applied in this study include choline chloride p.a. (Merck), fructose p.a. (Merck), lactic acid p.a. (Merck), glycerol p.a. (Merck), propylene glycol p.a. (Merck), proline p.a. (Merck), Folin-Ciocalteu reagent p.a. (Merck), carbonic acid p.a. (Merck), and gallic acid p.a. (Merck). In this context, the instrument utilized was a UV-Vis spectrophotometer (Shimadzu UV-1800).

2.1 Method

The study was conducted in 3 sections, namely sample preparation, NADESs preparation, and polyphenol extraction.

2.2 Sample preparation

The sample examined in this investigation was *S. acmella*, the various parts of which, including the stem, leaf, flower, and stalks were separated. The sample was dried at room temperature for a week without exposure to sunlight, before finely chopped and placed in plastic containers. Finally, the dried sample was stored at room temperature until further usage.

2.3 Preparation of NADESs

NADESs were mixed using a specific mole ratio of HBA and HBD. The choline chloride-based HBA compound was used with varied HBD compounds. It was important to acknowledge that the HBA mole comparison with HBD was 1:2. In this study, the HBD compounds included fructose, lactic acid, glycerol, propylene glycol, and proline. The preparation of NADESs comprised heating the mixtures containing solids and liquids, as well as solid-solid combinations, at a temperature of 150 °C while maintaining continuous stirring at 1400 rpm. The stirring process was conducted for a period ranging from 30 to 120 min until a clear solution was achieved [37]. Finally, the mixture was allowed to cool until room temperature was reached.

2.4 Extraction of active substance in *S. acmella* using NADES

The active substance from *S. acmella* was extracted by combining 1 g of dried sample with 10 mL of NADES in a glass beaker. Subsequently, stirring was conducted at 120 rpm and room temperature for 60 min. The supernatant was obtained by filtering the mixture through filter paper. Finally, the extracts were chemically analyzed using a spectrophotometer.

2.5 Determination of total phenolic content

The Folin-Ciocalteu method [38] was used to examine the overall content of phenols in the extract from *S. acmella* which was combined with distilled water in a ratio of 1:50 to form a solution. A total of 2 mL of Na_2CO_3 7.5% (w/v) was mixed with a combination of 500 µL extract and 2.5 mL of Folin-Ciocalteu reagent at a ratio of 1:10. The solution was allowed to incubate for 2 h at room temperature in a dark room. Subsequently, absorption was determined at a wavelength of 760 nm using a UV-Vis spectroscophotometer, by transferring 200 µL of the solution into a cuvet. Finally, gallic acid of 0–50 mg/L was applied to the standard solution and the yield was expressed in milligrams of gallic acid equivalent (GAE) per gram dried mass.

3 Result and discussion

3.1 Preparation of NADESs

Temperature, the ratio of solvent to liquid, and the extraction technique, as well as the physicochemical characteristics of the solvent utilized, contributed to the extraction capacity of phenolic compounds from botanical extracts. The physicochemical characteristics, including viscosity, polarity, physicochemical interactions, and solubility, were influenced by the chemical composition of the NADES compounds [2, 39, 40]. Furthermore, the process of extracting natural substances from food matrices are influenced by viscosity, polarity, density, and pH conditions, which were characterized as primary characteristics [8]. It was important to acknowledge that NADESs within the scope of this study, were synthesized from HBD compounds sourced from fructose (carbohydrate group–sugars), lactic acid (organic acid group), glycerol (polyalcohol group), propylene glycol (polyalcohol group), and proline (amino acid group), as well as HBA compounds derived from choline chloride, at mole ratio of 1:2. This process required 20% water to produce a clear NADESs and reduce the viscosity of the solution formed [14, 39, 41]. At room temperature, the resulting solvent formed a clear liquid solution, as detailed in Fig. 1.

NADESs were prepared using choline chloride as the HBA compound to enhance modification in terms of pH, viscosity, and polarity. This adaptability makes it suitable for various applications, including pharmaceuticals, food, and cosmetics [2, 42]. However, the resulting solvent had a weakness due to higher viscosity in contrast to water and other organic solvents [43]. The high viscosity of NADES is a major drawback in its industrial-scale applications [41, 44]. During the preparation process of this study, there was an additional 20% of water (w/w) to decrease the viscosity, thereby aiding the extraction process.

Introducing water as an additional constituent in the system can adjust the characteristics of NADES to enhance the extraction of various chemicals and substances, particularly for applications in food testing [8]. According to Dai et al. [45], adding water to the solution led to a reduction in the viscosity of the solvent and a weakening of the hydrogen bond interactions among the constituents. However, the dilution below 50% usually does not cause matrix interference in any of the components. As mentioned by Mitar et al. [46], Ivanović et al. [41], and Pires et al. [44], the viscosity and density of the NADES can be decreased by adding water to the solution. However, this needs to be done carefully because too much water can disrupt hydrogen bonding

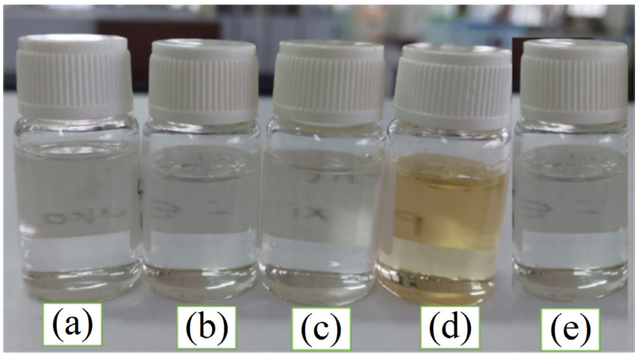


Fig. 1 The NADESs based on (a) choline chloride-fructose; (b) choline chloride-lactic acid; (c) choline chloride-glycerol; (d) choline chloride-propylene glycol; (e) choline chloride-proline

and eliminate eutectic properties. The addition of water typically ranges from 20% – 40% (w/w), hence, approximately 20% was added in this study. Rosarina et al. [22] stated that the water addition to the solvent constituted of choline chloride-lactic acid (1:2) provided an increased curcuminoid yield from 25% to 35% (w/w). Table 1 presents the characteristics of synthesized NADESs and the mole ratio used.

The properties of the synthesized NADESs such as the polarity and the affinity of extraction can be modified by adjusting the molar ratio of their constituents [5]. Cao et al. [47] stated that the ability of NADES to dissolve target compounds can be modified by adjusting the type of NADES constituents, as well as the molar ratio of the components and the water content. The molar comparison of HBA and HBD affects the properties of the solvent produced. Therefore, a 1:2 mole ratio of HBA to HBD was adopted. Similar comparisons have also been made by Islamčević Razboršek et al. [40], indicating that NADES derived from choline chloride and lactic acid at a mole ratio of 1:2 led to an increased extraction yield in contrast to 1:1. This occurred due to the utilization of NADES derived from choline chloride and lactic acid (1:2), which had a lower viscosity and higher polarity, leading to better extraction compared to the ratio of 1:1 [48]. A similar comparison was also conducted on phenolic extraction from peppermint Jurić et al. [49] who showed the best extraction efficiency yields.

3.2 Polyphenol extraction from *S. acmella*

The prepared NADESs were used for extracting polyphenol compounds from the stems, stalks, leaves, and flowers of *S. acmella*, presented in Fig. 2. The resulting extract had a distinctive color, as detailed in Fig. 3.

The levels of polyphenols in extracts of flowers, stalks, leaves, and stems were analyzed using the Folin-Ciocalteu method with gallic acid as the control substance. Table 2 summarizes the results of the polyphenol component extraction from the plant using the developed NADESs.

Table 2 shows that ChCl-Fr was the best solvent for extracting polyphenolic compounds from the stalks, leaves, and stems of *S. acmella*. Meanwhile, on the flower part, the best solvent is ChCl-Gly. The highest concentration of polyphenols of 2545.192 mg/L was observed to be extracted from the stems of *S. acmella*. The application of NADES as a solvent used to extract polyphenol was compared with conventional solvents in a previous study. Islamčević Razboršek et al. [40] show that NADES based on choline chloride had higher extraction capabilities than

Table 1 NADESs studied and their characteristics

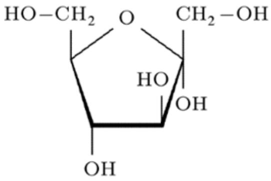
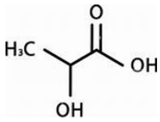
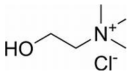
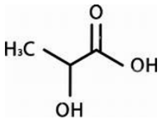
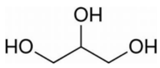
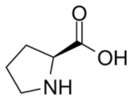
Type of NADESs	Components		Abbreviation	Mole ratio	Visual
	HBA	HBD			
NADES 1		Fructose 	ChCl-Fr	1:2	Clear colorless liquid, transparent, slightly thick
NADES 2		Lactic acid 	ChCl-LA	1:2	Clear colorless liquid, transparent, slightly thick
NADES 3	Choline chloride 	Glycerol 	ChCl-Gly	1:2	Clear colorless liquid, transparent, slightly thick
NADES 4		Propylene glycol 	ChCl-PG	1:2	Clear colored liquid, slightly thick
NADES 5		Proline 	ChCl-Pro	1:2	Clear colorless liquid, slightly thick

Fig. 2 The parts of the *S. acmella* extracted with NADES: (a) flowers; (b) stalks; (c) stems; (d) leaves

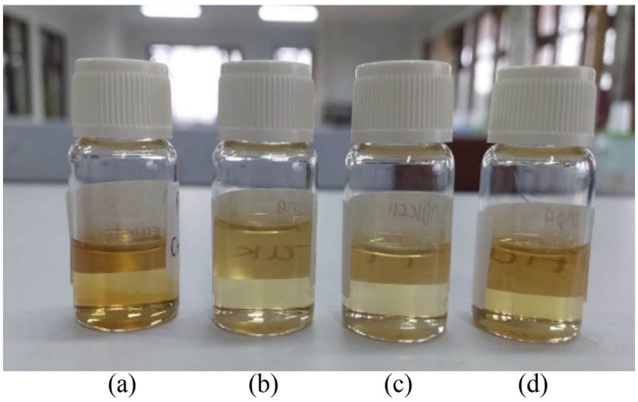


Fig. 3 The results of polyphenol extracts in NADES based on choline chloride and fructose: (a) leaves; (b) stems; (c) stalks; (d) flowers

conventional methanolic extraction (with 80% MeOH). According to Chu et al. [50], the extraction efficiency of polyphenols using NADES was 1.68 times greater than 70% ethanol. Similarly, Gómez-Urios et al. [51] stated that the solvent had greater extraction yields than 50% (v/v) ethanol. For these reasons, NADES has great potential that can be utilized in the extraction of polyphenols.

The result of the extraction of polyphenolic compounds in *S. acmella* was quantified in milligrams of gallic acid equivalent (GAE) per gram of the dried extract, as presented in Fig. 4. The term "extraction yield" refers to the levels of polyphenolic compounds extracted using the solvent. NADESs based on choline chloride with variations

Table 2 Polyphenol concentrations in NADES

Solvents	Polyphenol concentrations (mg/L)			
	Flowers	Stalks	Leaves	Stems
ChCl-Fr	205.288	311.058	628.365	2545.192
ChCl-LA	68.796	81.759	77.593	154.907
ChCl-Gly	241.509	187.736	422.170	258.019
ChCl-PG	90.566	68.811	144.340	91.038
ChCl-Pro	175.000	84.722	480.556	40.741

of HBD among others fructose, lactic acid, glycerol, propylene glycol, or proline were utilized for the extraction process. Results showed that the yield of the extraction in case of the flower of the *S. acmella* was 2.053 mg GAE/g, 0.688 mg GAE/g, 2.415 mg GAE/g, 0.906 mg GAE/g, and 1.750 mg GAE/g, respectively. This suggested ChCl-Gly as the best solvent for polyphenol extraction from *S. acmella* flower, with the yield being 2.415 mg GAE/g extract. The extraction efficiency of *S. acmella* flower was obtained using NADESs in the sequence ChCl-Gly > ChCl-Fr > ChCl-Pro > ChCl-PG > ChCl-LA.

ChCl-Fr showed superior effectiveness compared to others, in extracting polyphenol compounds from the stalks, leaves, and stems of *S. acmella*. The highest polyphenol yield in the stalks was 3.111 mg GAE/g dry extract. The polyphenol extraction efficiency of *S. acmella* stalks was ChCl-Fr > ChCl-Gly > ChCl-Pro > ChCl-PG > ChCl-LA.

In the leaves of *S. acmella*, the optimum polyphenol yield that can be extracted was 6.284 mg GAE/g dry extract. This was achieved using NADESs, characterized by ChCl-Fr > ChCl-Pro > ChCl-Gly > ChCl-PG > ChCl-LA. The extraction yield of polyphenol from the stems was much higher than from other parts of *S. acmella*, reaching 25.452 mg GAE/g dry extract. The order of effectiveness of extracting polyphenols from the stems using NADESs was ChCl-Fr > ChCl-Gly > ChCl-LA > ChCl-PG > ChCl-Pro. The excellent performance of NADES may be attributed to the hydrophilic nature of the phenolic compounds, which enhances the polarity of choline chloride as well as of sugars, polyalcohols, and organic acids. The high extraction ability of the solvent was associated with the interaction of hydrogen bonding between NADES molecules and polyphenolic compounds [52]. The extract derived from NADES 1 (ChCl-Fr) had the highest concentrations of polyphenols. This was in line with the study conducted by Islamčević Razboršek et al. [40] on the extraction of polyphenols from chokeberry (*Aronia melanocarpa*). The polyphenol extraction yield from *S. acmella* flower was 2.415 mg GAE/g. The order of extraction efficiency of polyphenols from the flower of *S. acmella* with the formulations with choline chloride was glycerol > fructose > proline > propylene glycol > lactic acid. Therefore, the most effective solvent for extracting polyphenols from the stalks, stems, and leaves of *S. acmella* is NADES 1, which is composed of choline chloride and fructose. Meanwhile, NADES 3 (ChCl-Gly) was positioned as the optimal solvent for polyphenol extraction from the flower of *S. acmella*.

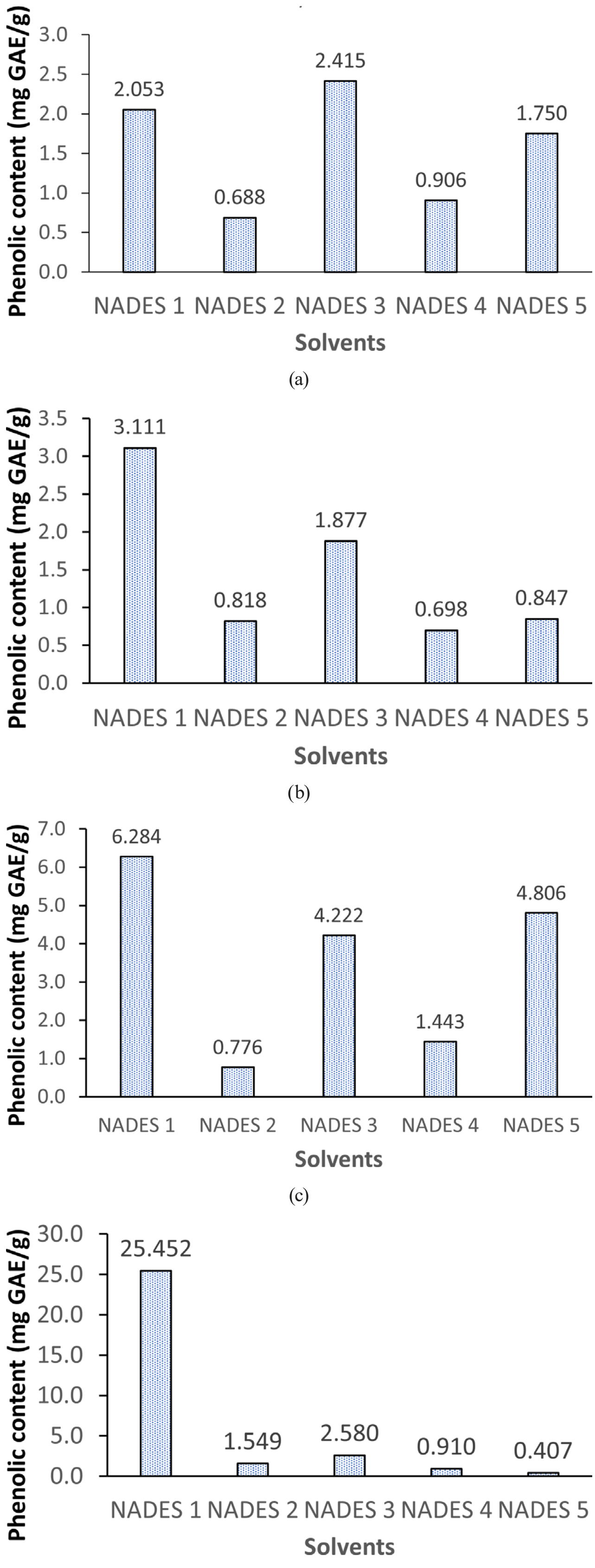


Fig. 4 The results of extracting polyphenol compounds from *S. acmella* using NADES; (a) flower; (b) stalks; (c) leaves; (d) stems

The ability of NADES to extract polyphenols was influenced by several factors, including polarity, viscosity, solubility, and physicochemical interactions, in particular hydrogen bonds between the solvent and the active compound [53]. Phenolic compunds are secondary metabolites of plants, consisting of a phenol structure of aromatic rings with one or more hydroxyl groups (−OH) [39, 54]. The highest levels of polyphenols were discovered in the stems of the *S. acmella*, in the order ChCl-Fr > ChCl-Gly > ChCl-LA> ChCl-PG > ChCl-Pro. This can be explained by the theory of "like dissolves like", where polar compounds dissolve well in polar solvents and *vice versa*. Polyphenols are phenolic compounds possess OH groups, hence, they are polar compounds. Among the 5 HBDs used, proline was the compound with the lowest polarity. It is essential to recognize that the extraction ability of ChCl-Pro was the lowest. Furthermore, since glycerol was more polar than propylene glycol, the polyphenol extraction ability of ChCl-Gly was greater than that of ChCl-PG and ChCl-Pro. This polarity factor majorly influences the extraction ability of active compounds, as stated by Mohd Fuad et al. [55] and Meenu et al. [56]. However, the extraction of polyphenols from plants using NADESs is a complicated process influenced by several factors, such as polarity, H-bond interaction, temperature, solid/liquid ratio, addition of water, and viscosity [54, 57]. Results showed that the highest polyphenol extraction ability was in ChCl-Fr. This is possibly due to hydrogen bond interactions. As observed, fructose has more hydrogen bond interactions between the solvent and polyphenols, leading to a greater yield [57]. Furthermore, the difference in the amount of extract produced in various parts of *S. acmella* was attributed to each part containing different active compounds. As a results, the matrix in the sample interacts with solvent used.

4 Conclusion

In conclusion, NADES was a highly potential solvent as a green solvent for extracting polyphenol compounds from *S. acmella*. In this study, the composition of choline chloride and glycerol in a ratio of 1:2 was considered optimal for extraction process of *S. acmella*'s flower. The stalk, stem, and leaf of *S. acmella* showed the highest extraction efficiency with NADES derived from choline chloride and fructose. The extraction capacity was attributed to the hydrogen bond interaction between NADES and polyphenolic compounds in the plant. Furthermore, the effectiveness of NADES in extraction was affected by the physicochemical characteristics, which depend on the mole ratio of the constituent components. Further investigation was needed to examine the extraction of other plants using NADES, as well as in the development in the fields of food, pharmaceuticals, and health.

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References

[1] WHO "Annex 1: WHO guidelines on good herbal processing practices for herbal medicines", World Health Organization, Geneva, Switzerland, Rep. 1010, 2018.

[2] Hikmawanti, N. P. E., Ramadon, D., Jantan, I., Mun'im, A. "Natural Deep Eutectic Solvents (NADES): Phytochemical Extraction Performance Enhancer for Pharmaceutical and Nutraceutical Product Development", Plants, 10(10), 2091, 2021. https://doi.org/10.3390/plants10102091

[3] Alañón, M. E., Ivanović, M., Gómez-Caravaca, A. M., Arráez-Román, D., Segura-Carretero, A. "Choline chloride derivative-based deep eutectic liquids as novel green alternative solvents for extraction of phenolic compounds from olive leaf", Arabian Journal of Chemistry, 13(1), pp. 1685–1701, 2020. https://doi.org/10.1016/j.arabjc.2018.01.003

[4] Abubakar, A. R., Haque, M. "Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes", Journal of Pharmacy & BioAllied Sciences, 12(1), pp. 1–10, 2020. https://doi.org/10.4103/jpbs.JPBS_175_19

[5] Bajkacz, S., Adamek, J. "Development of a Method Based on Natural Deep Eutectic Solvents for Extraction of Flavonoids from Food Samples", Food Analytical Methods, 11(5), pp. 1330–1344, 2018. https://doi.org/10.1007/s12161-017-1118-5

[6] Diuzheva, A., Carradori, S., Andruch, V., Locatelli, M., De Luca, E., Tiecco, M., Germani, R., Menghini, L., Nocentini, A., Gratteri, P., Campestre, C. "Use of Innovative (Micro)Extraction Techniques to Characterise Harpagophytum procumbens Root and its Commercial Food Supplements", Phytochemical Analysis, 29(3), pp. 233–241, 2018. https://doi.org/10.1002/pca.2737

[7] Popovic, B. M., Micic, N., Potkonjak, A., Blagojevic, B., Pavlovic, K., Milanov, D., Juric, T. "Novel extraction of polyphenols from sour cherry pomace using natural deep eutectic solvents – Ultrafast microwave-assisted NADES preparation and extraction", Food Chemistry, 366, 130562, 2022. https://doi.org/10.1016/j.foodchem.2021.130562

[8] Paiva, A., Craveiro, R., Aroso, I., Martins, M., Reis, R. L., Duarte, A. R. C. "Natural Deep Eutectic Solvents – Solvents for the 21st Century", ACS Sustainable Chemistry & Engineering, 2(5), pp. 1063–1071, 2014.
https://doi.org/10.1021/sc500096j

[9] Płotka-Wasylka, J., de la Guardia, M., Andruch, V., Vilková, M. "Deep eutectic solvents vs ionic liquids: Similarities and differences", Microchemical Journal, 159, 105539, 2020.
https://doi.org/10.1016/j.microc.2020.105539

[10] Myrdek, T., Popescu, C., Kunz, W. "Physical-chemical properties of newly synthesized tetraalkylammonium alkyl ether carboxylate ionic liquids", Journal of Molecular Liquids, 322, 114947, 2021.
https://doi.org/10.1016/j.molliq.2020.114947

[11] Mamusa, M., Chelazzi, D., Baglioni, M., Murgia, S., Fratini, E., Rivillo, D., van Leeuwen, P. W. N. M., Schrekker, H. S., Baglioni, P. "[BMI*m*][BARF] imidazolium salt solutions in alkyl carbonate solvents: Structure and interactions", Applied Materials Today, 31, 101741, 2023.
https://doi.org/10.1016/j.apmt.2023.101741

[12] Rashid, T. U. "Ionic liquids: Innovative fluids for sustainable gas separation from industrial waste stream", Journal of Molecular Liquids, 321, 114916, 2021.
https://doi.org/10.1016/j.molliq.2020.114916

[13] Prabhune, A., Dey, R. "Green and sustainable solvents of the future: Deep eutectic solvents", Journal of Molecular Liquids, 379, 121676, 2023.
https://doi.org/10.1016/j.molliq.2023.121676

[14] Flieger, J., Flieger, M. "Ionic Liquids Toxicity—Benefits and Threats", International Journal of Molecular Sciences, 21(17), 6267, 2020.
https://doi.org/10.3390/ijms21176267

[15] Cho, C.-W., Pham, T. P. T., Zhao, Y., Stolte, S., Yun, Y.-S. "Review of the toxic effects of ionic liquids", Science of The Total Environment, 786, 147309, 2021.
https://doi.org/10.1016/j.scitotenv.2021.147309

[16] Dzhavakhyan, M. A., Prozhogina, Y. E. "Deep Eutectic Solvents: History, Properties, and Prospects", Pharmaceutical Chemistry Journal, 57(2), pp. 296–299, 2023.
https://doi.org/10.1007/s11094-023-02879-0

[17] Nian, B., Li, X. "Can deep eutectic solvents be the best alternatives to ionic liquids and organic solvents: A perspective in enzyme catalytic reactions", International Journal of Biological Macromolecules, 217, pp. 255–269, 2022.
https://doi.org/10.1016/j.ijbiomac.2022.07.044

[18] Veríssimo, N. V. P., Mussagy, C. U., Bento, H. B. S., Pereira, J. F. B., Santos-Ebinuma, V. d. C. "Ionic liquids and deep eutectic solvents for the stabilization of biopharmaceuticals: A review", Biotechnology Advances, 71, 108316, 2024.
https://doi.org/10.1016/j.biotechadv.2024.108316

[19] Sanati, A., Malayeri, M. R., Busse, O., Weigand, J. J. "Utilization of ionic liquids and deep eutectic solvents in oil operations: Progress and challenges", Journal of Molecular Liquids, 361, 119641, 2022.
https://doi.org/10.1016/j.molliq.2022.119641

[20] Siamandoura, P., Tzia, C. "Comparative Study of Novel Methods for Olive Leaf Phenolic Compound Extraction Using NADES as Solvents", Molecules, 28(1), 353, 2023.
https://doi.org/10.3390/molecules28010353

[21] Elgharbawy, A. A. M., Hayyan, A., Hayyan, M., Mirghani, M. E. S., Salleh, H. M., Rashid, S. N., Ngoh, G. C., Liew, S. Q., Nor, M. R. M., bin Mohd Yusoff, M. Y. Z., Alias, Y. "Natural Deep Eutectic Solvent-Assisted Pectin Extraction from Pomelo Peel Using Sonoreactor: Experimental Optimization Approach", Processes, 7(7), 416, 2019.
https://doi.org/10.3390/pr7070416

[22] Rosarina, D., Narawangsa, D. R., Chandra, N. S. R., Sari, E., Hermansyah, H. "Optimization of Ultrasonic—Assisted Extraction (UAE) Method Using Natural Deep Eutectic Solvent (NADES) to Increase Curcuminoid Yield from *Curcuma longa* L., *Curcuma xanthorrhiza*, and *Curcuma mangga* Val.", Molecules, 27(18), 6080, 2022.
https://doi.org/10.3390/molecules27186080

[23] Oomen, W. W., P. Begines, P., Mustafa, N. R., Wilson, E. G., Verpoorte, R, Choi, Y. H. "Natural Deep Eutectic Solvent Extraction of Flavonoids of *Scutellaria baicalensis* as a Replacement for Conventional Organic Solvents", Molecules, 25(3), 617, 2020.
https://doi.org/10.3390/molecules25030617

[24] Vo, T. P., Pham, N. G., Pham, T. V., Nguyen, H. Y., Vo, L. T. V., Tran, T. N. H., Tran, T. N., Nguyen,D. Q. "Green extraction of total phenolic and flavonoid contents from mangosteen (*Garcinia mangostana* L) rind using natural deep eutectic solvents", Heliyon, 9(4), e14884, 2023.
https://doi.org/10.1016/j.heliyon.2023.e14884

[25] Chen, Z., Ragauskas, A., Wan, C. "Lignin extraction and upgrading using deep eutectic solvents", Industrial Crops and Products, 147, 112241, 2020.
https://doi.org/10.1016/j.indcrop.2020.112241

[26] Pavlić, B., Mrkonjić, Ž., Teslić, N., Kljakić, A. C., Pojić, M., Mandić, A., Stupar, A., Santos, F., Duarte, A. R. C., Mišan, A. "Natural Deep Eutectic Solvent (NADES) Extraction Improves Polyphenol Yield and Antioxidant Activity of Wild Thyme (*Thymus serpyllum* L.) Extracts", Molecules, 27(5), 1508, 2022.
https://doi.org/10.3390/molecules27051508

[27] Sachdev, M., Tirupathi, S. P., Afnan, L. "Clinical Uses and Scope of *Spilanthes acmella* in Dentistry: A Narrative Review", Journal of Natural Remedies, 23(2), pp. 397–402, 2023.
https://doi.org/10.18311/jnr/2023/33016

[28] Pradhan, N. R., Mishra, K. G., Patnaik, N., Nayak, R. "Evaluation of effects of Spilanthes acmella extract on muscle mass and sexual potency in males: A population-based study", Journal of Family Medicine and Primary Care, 10(11), pp. 4242–4246, 2021.
https://doi.org/10.4103/jfmpc.jfmpc_746_21

[29] De, B., Goswami, T. K. "Chapter 32 - Spilanthol (affinin)", In: Mushtaq, M., Anwar, F. (eds.) A Centum of Valuable Plant Bioactives, Academic Press, 2021, pp. 733–743. ISBN 978-0-12-822923-1
https://doi.org/10.1016/B978-0-12-822923-1.00030-3

[30] Singh, M., Chaturvedi, R. "Screening and quantification of an antiseptic alkylamide, spilanthol from in vitro cell and tissue cultures of *Spilanthes acmella* Murr.", Industrial Crops and Products, 36(1), pp. 321–328, 2012.
https://doi.org/10.1016/j.indcrop.2011.10.029

[31] Dias, A. M. A, Santos, P., Seabra,I. J., Junior, R. N. C., Braga, M. E. M., de Sousa, H. C. "Spilanthol from *Spilanthes acmella* flowers, leaves and stems obtained selective supercritical carbon dioxide extraction", The Journal of Supercritical Fluids, 61, pp. 62–70, 2012.
https://doi.org/10.1016/j.supflu.2011.09.020

[32] Barbosa, A. F., Pereira, C. D. S. S., Mendes, M. F., De Carvalho Junior, R. N., De Carvalho, M. G., Maia, J. G. S., Sabaa-Srur, A. U. O. "Spilanthol Content in the Extract Obtained by Supercritical CO_2 at Different Storage Times of Acmella Oleracea L.", Journal of Food Process Engineering, 40(3), e12441, 2017.
https://doi.org/10.1111/jfpe.12441

[33] Nakatani, N., Nagashima, M. "Pungent Alkamides from *Spilanthes acmella* L. var. oleracea Clarke", Bioscience, Biotechnology & Biochemistry, 56(5), pp. 759–762, 1992.
https://doi.org/10.1271/bbb.56.759

[34] Moreno, S. C., Carvalho, G. A., Picanço, M. C., Morais, E. G. F., Pereira, R. M. "Bioactivity of compounds from *Acmella oleracea* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and selectivity to two non-target species", Pest Management Science, 68(3), pp. 386–393. 2012.
https://doi.org/10.1002/ps.2274

[35] Nabi, N. G., Shrivastava, M. "Estimation of Total Flavonoids and Antioxidant Activity of Spilanthes acmella Leaves", Pharmaceutical and Biosciences Journal, 4(6), pp. 29–34, 2016.
https://doi.org/10.20510/ukjpb/4/i6/134657

[36] Uthpala, T. G. G., Navaratne, S. B. "*Acmella oleracea* Plant; Identification, Applications and Use as an Emerging Food Source – Review", Food Reviews International, 37(4), pp. 399–414, 2021.
https://doi.org/10.1080/87559129.2019.1709201

[37] Dai, Y., Row, K. H. "Application of Natural Deep Eutectic Solvents in the Extraction of Quercetin from Vegetables", Molecules, 24(12), 2300, 2019.
https://doi.org/10.3390/molecules24122300

[38] Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., Koirala, N. "Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal", Plants, 8(4), 96, 2019.
https://doi.org/10.3390/plants8040096

[39] Koh, Q. Q., Kua, Y. L.,Gan, S., Tan, K. W., Lee, T. Z. E., Cheng, W. K., Lau, H. L. N. "Sugar-based natural deep eutectic solvent (NADES): Physicochemical properties, antimicrobial activity, toxicity, biodegradability and potential use as green extraction media for phytonutrients", Sustainable Chemistry and Pharmacy, 35, 101218, 2023.
https://doi.org/10.1016/j.scp.2023.101218

[40] Islamčević Razboršek, M., Ivanović, M., Krajnc, P., Kolar, M. "Choline Chloride Based Natural Deep Eutectic Solvents as Extraction Media for Extracting Phenolic Compounds from Chokeberry (*Aronia melanocarpa*)", Molecules, 25(7), 1619, 2020.
https://doi.org/10.3390/molecules25071619

[41] Ivanović, M., Islamčević Razboršek, M., Kolar, M. "Innovative Extraction Techniques Using Deep Eutectic Solvents and Analytical Methods for the Isolation and Characterization of Natural Bioactive Compounds from Plant material", Plants, 9(11), 1428, 2020.
https://doi.org/10.3390/plants9111428

[42] Choi, Y. H., Verpoorte, R. "Green solvents for the extraction of bioactive compounds from natural products using ionic liquids and deep eutectic solvents", Current Opinion in Food Science, 26, pp. 87–93, 2019.
https://doi.org/10.1016/j.cofs.2019.04.003

[43] Liu, X., Fu, N., Zhang, Q., Cai, S., Wang, Q., Han, D., Tang, B. "Green Tailoring With Water of Choline Chloride Deep Eutectic Solvents for the Extraction of Polyphenols From Palm Samples", Journal of Chromatographic Science, 57(3), pp. 272–278, 2019.
https://doi.org/10.1093/chromsci/bmy099

[44] Pires, I. V., Sakurai, Y. C. N., Ferreira, N. R., Moreira, S. G. C., da Cruz Rodrigues, A. M., da Silva, L. H. M. “Elaboration and Characterization of Natural Deep Eutectic Solvents (NADESs): Application in the Extraction of Phenolic Compounds from *pitaya*”, Molecules, 27(23), 8310, 2022.
https://doi.org/10.3390/molecules27238310

[45] Dai, Y., Witkamp, G.-J., Verpoorte, R., Choi, Y. H. "Tailoring properties of natural deep eutectic solvents with water to facilitate their applications", Food Chemistry, 187, pp. 14–19, 2015.
https://doi.org/10.1016/j.foodchem.2015.03.123

[46] Mitar, A., Panić, M., Kardum, J. P., Halambek, J., Sander, A., Kučan, K. Z., Radojčić Redovniković, I., Radošević, K. "Physicochemical Properties, Cytotoxicity, and Antioxidative Activity of Natural Deep Eutectic Solvents Containing Organic Acid", Chemical and Biochemical Engineering Quarterly, 33(1), pp. 1–18, 2019.
https://doi.org/10.15255/CABEQ.2018.1454

[47] Cao, J., Cao, J., Wang, H., Chen, L., Cao, F., Su, E. "Solubility improvement of phytochemicals using (natural) deep eutectic solvents and their bioactivity evaluation", Journal of Molecular Liquids, 318, 113997, 2020.
https://doi.org/10.1016/j.molliq.2020.113997

[48] Bakirtzi, C., Triantafyllidou, K., Makris, D. P. "Novel lactic acid-based natural deep eutectic solvents: Efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from common native Greek medicinal plants", Journal of Applied Research on Medicinal and Aromatic Plants, 3(3), pp. 120–127, 2016.
https://doi.org/10.1016/j.jarmap.2016.03.003

[49] Jurić, T., Mićić, N., Potkonjak, A., Milanov, D., Dodić, J., Trivunović, Z., Popović, B. M. "The evaluation of phenolic content, *in vitro* antioxidant and antibacterial activity of *Mentha piperita* extracts obtained by natural deep eutectic solvents", Food Chemistry, 362, 130226, 2021.
https://doi.org/10.1016/j.foodchem.2021.130226

[50] Chu, J., Ming, Y., Cui, Q., Yang, S., Li, W., Gao, H., Zhang, R., Cheng, X. "Efficient extraction and antioxidant activity of polyphenols from *Antrodia cinnamomea*", BMC Biotechnology, 22(1), 9, 2022.
https://doi.org/10.1186/s12896-022-00739-5

[51] Gómez-Urios, C., Viñas-Ospino, A., Puchades-Colera, P., Blesa, J., López-Malo, D., Frígola, A., Esteve, M. J. "Choline chloride-based natural deep eutectic solvents for the extraction and stability of phenolic compounds, ascorbic acid, and antioxidant capacity from *Citrus sinensis* peel", LWT, 177, 114595, 2023. https://doi.org/10.1016/j.lwt.2023.114595

[52] Zannou, O., Koca, I. "Greener extraction of anthocyanins and antioxidant activity from blackberry (*Rubus* spp) using natural deep eutectic solvents", LWT, 158, 113184, 2022. https://doi.org/10.1016/j.lwt.2022.113184

[53] Rente, D., Paiva, A., Duarte, A. R. "The Role of Hydrogen Bond Donor on the Extraction of Phenolic Compounds from Natural Matrices Using Deep Eutectic Systems", Molecules, 26(8), 2336, 2021. https://doi.org/10.3390/molecules26082336

[54] Dheyab, A. S., Abu Bakar, M. F., AlOmar, M., Sabran, S. F., Muhamad Hanafi, A. F., Mohamad, A. "Deep Eutectic Solvents (DESs) as Green Extraction Media of Beneficial Bioactive Phytochemicals", Separations, 8(10), 176, 2021. https://doi.org/10.3390/SEPARATIONS8100176

[55] Mohd Fuad, F., Mohd Nadzir, M., Harun@Kamaruddin, A. "Hydrophilic natural deep eutectic solvent: A review on physicochemical properties and extractability of bioactive compounds", Journal of Molecular Liquids, 339, 116923, 2021. https://doi.org/10.1016/j.molliq.2021.116923

[56] Meenu, M., Bansal, V., Rana, S., Sharma, N., Kumar, V., Arora, V., Garg, M. "Deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESs): Designer solvents for green extraction of anthocyanin", Sustainable Chemistry Pharmacy, 34, 101168, 2023. https://doi.org/10.1016/j.scp.2023.101168

[57] Aktaş, H., Kurek, M. A. "Deep eutectic solvents for the extraction of polyphenols from food plants", Food Chemistry, 444, 138629, 2024. https://doi.org/10.1016/j.foodchem.2024.138629