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Natural deep eutectic solvents: promising agents for extracting substances from plant materials

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ABSTRACT

There is a growing trend to replace synthetic products or additives with natural alternatives. Historical experience and developments in human health science demonstrate the adverse effects of xenobiotics on human health and metabolism. The shift toward natural products is essential for enhancing vitality, social activity, and overall quality of life, while preventing adverse events and diseases. Industries such as pharmaceutical, cosmetic, and food are increasingly turning to natural and biologically active chemicals isolated from plants or microorganisms. However, the primary challenge lies in developing effective and eco-friendly methods for their isolation. Deep eutectic solvents are a mixture of two or more components with unique properties like low volatility, low melting points, ease of preparation, cost-effective starting materials, and minimal toxicity to humans. They have gained popularity as environmentally friendly agents for extracting biologically active substances from medicinal plants and as safe alternatives for various industries, including food and pharmaceuticals. Traditional organic solvent extraction methods have several drawbacks, including long extraction times, safety concerns, environmental damage, high cost, and the need for large solvent volumes. This review summarizes research progress on the benefits of using deep eutectic solvents for extracting bioactive compounds, including phenolic acid, flavonoids, isoflavones, catechins, polysaccharides, curcuminoids, proanthocyanidin, phycocyanin, gingerols, ginsenosides, anthocyanins, rutin, and chlorogenic acids. Additionally, it examines the biological activity of these extracts, including antioxidant, antibacterial, and antitumor properties.

Keywords: biologically active compounds; extraction; deep eutectic solvents; antioxidant activity; antitumor activity; antibacterial activity; flavonoids; alkaloids; polyphenols.

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Природные глубокие эвтектические растворители – перспективные агенты для экстракции веществ из растительного сырья

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АННОТАЦИЯ

В последнее время неуклонно возрастает тенденция к сокращению количества синтетических продуктов или добавок и замене их натуральными. Исторический опыт и развитие науки о здоровье человека располагают убедительными данными о неблагоприятных эффектах ксенобиотиков на естественный метаболизм и здоровье человека в целом. Максимальная натурализация пищевых и бытовых продуктов широкого потребления является значимым в поддержании адекватными требованиям среды обитания жизнеспособности, социальной активности, достойного качества жизни, профилактике нежелательных явлений и болезней. Фармацевтическая, косметическая и пищевая промышленность уделяют особое внимание натуральным и биологически активным химическим веществам, выделенным из растений или микроорганизмов. Основная задача в этом направлении — разработка эффективных и экологических методов их выделения. Глубокие эвтектические растворители представляют собой смесь двух или более компонентов и относятся к растворителям с уникальными свойствами, такими как низкая летучесть, низкие температуры плавления, простота приготовления, недорогие исходные вещества и, самое главное, они практически нетоксичны для человека. Глубокие эвтектические растворители используются в качестве экологичного метода экстракции биологически активных веществ из лекарственных растений, а также в качестве безопасной альтернативы для пищевых, фармацевтических и различных отраслей промышленности. Традиционные методы экстракции органическими растворителями имеют ряд недостатков, таких как длительный период экстракции, безопасность их использования, урон для окружающей среды, высокая стоимость и необходимость использования больших объемов растворителей. В этом обзоре представлено краткое описание прогресса исследований, касающихся преимуществ использования глубоких эвтектических растворителей для экстракции биоактивных соединений, таких как фенольная кислота, флавоноиды, изофлавоны, катехины, полисахариды, куркуминоиды, проантоцианидин, фикоцианин, гингеролы, гинсенозиды, антоцианы, рутин, хлорогеновые кислоты и др. Рассмотрено изучение биологической активности экстрактов — антиоксидантной, антибактериальной и противоопухолевой активности.

Ключевые слова: биологически активные соединения; экстракция; глубокие эвтектические растворители; антиоксидантная активность; противоопухолевая активность; антибактериальная активность; флавоноиды; алкалоиды; полифенолы.

Как цитировать

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INTRODUCTION

The World Health Organization reports that approximately 80% of the global population uses medicinal herbs for the treatment of various diseases and conditions. The biologically active substances contained in most plants, including polyphenols, flavonoids, alkaloids, polysaccharides, carotenoids, azadirachtin, aloin, and ginsenosides, have a significant positive effect on human health. These substances have a wide range of pharmacological effects, including antimicrobial, anti-inflammatory, antioxidant, vasoactive, neuroprotective, and antithrombotic properties [1]. Natural bioactive compounds are commonly extracted from the roots, leaves, and fruits of plants, as well as algae and fungi. Various bioactive components require different extraction methods, and their activities also vary with the extraction methods and conditions.

Extraction methods are classified into two primary categories: traditional and alternative. Traditional methods include percolation, maceration, and Soxhlet extraction. Alternative methods involve microwave extraction, ultrasonic extraction, and enzymatic extraction [2, 3] (Fig. 1).

The extraction of bioactive compounds from plants is typically performed using various aqueous organic solvents, including hexane, methanol, benzene, chloroform, petroleum ether, and acetone. Alternative methods are more efficient than traditional methods, with the extraction process taking less time, being less costly, and giving higher purity of the extracted compounds. Both types of extraction methods have a number of limitations. These include the toxicity of traditional solvents, which is a major concern in light of "residual solvents"; thermal instability, which can affect the subsequent extraction of bioactive compounds from solvents, and the

potential impact on the chemical structure of bioactive compounds.

Thus, the need for green solvents for use in various industries is increasing annually. Green solvents should have important characteristics such as nonflammability, thermal stability, chemical safety, low volatility, and low toxicity. Therefore, the use of deep eutectic solvents in the extraction of bioactive compounds is an environmentally friendly and progressive method. Deep eutectic solvents (DESs) of natural origin are based on a combination of essential metabolites, such as alcohols, sugars, amino acids, and organic acids. Currently, there are numerous studies investigating the selectivity of DESs in the extraction of bioactive compounds, such as flavonoids, polyphenols, phenolic acids, saponins, and anthraquinones from various natural sources [4].

This review summarizes the role of the most important groups of biologically active plant compounds, namely, phenols, flavonoids, terpenes, polysaccharides, and others. The focus is on new discoveries in their biological activity, potential applications of extracted chemicals, and environmentally safe and efficient extraction methods using DESs.

DESS: A BRIEF REVIEW

Abbott et al. [5] were the first to synthesize a DES based on choline chloride and urea. The synthesis of DESs typically involves gently heating a mixture of two solids while stirring to form clear liquid solution that is stable at room temperature. One of the substances acts as a hydrogen bond acceptor, while the other acts as a hydrogen bond donor. Potential hydrogen bond acceptors include choline chloride, acetylcholine chloride, and various amino acids [6]. Potential hydrogen bond donors include amines (urea), organic acids (lactic acid, citric

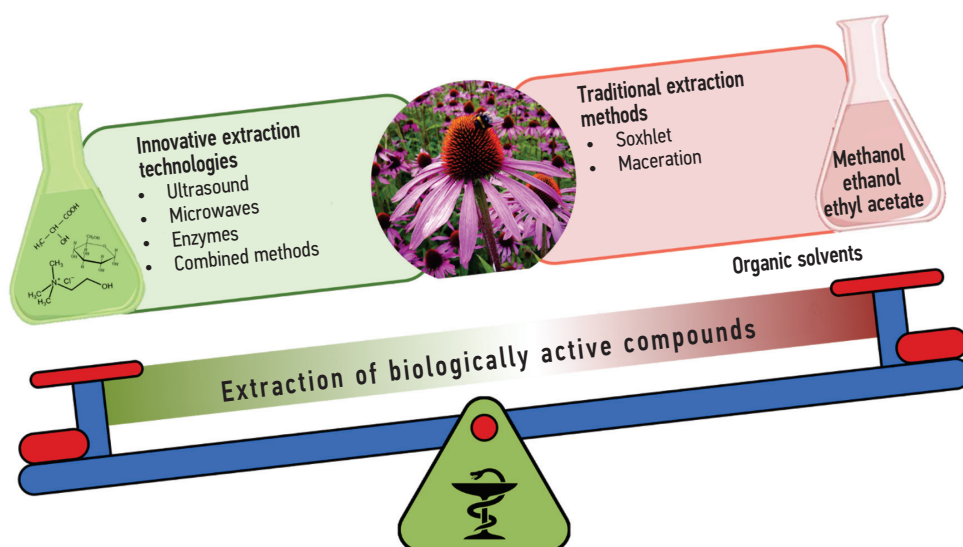


Fig. 1. Methods for extracting biologically active substances from plants

Рис. 1. Методы извлечения биологически активных веществ из растений

acid, and tartaric acid). DESs have several advantages, including the ready availability and low cost of their ingredients, as well as a simple and rapid synthesis process. However, some studies have indicated that various DESs may be toxic and cytotoxic. For instance, the cytotoxicity of certain phosphonium based DESs is higher than that of the individual components of their formulations, and the overall toxicity of DESs varies with the structures of the starting substances [7]. Therefore, it is possible to alter the composition of DESs to achieve the desired physicochemical properties, biological effects, and toxicological profiles. Choi et al. [8] proposed the concept of natural DESs (NDEs), which consist exclusively of natural components, namely, primary metabolites (e. g., sugars, amino acids, organic acids, polyols, and tertiary amines) [8]. They posed the question of why certain simple substances are consistently present in significant quantities in all microorganisms, as well as in the mammalian and plant kingdoms. These compounds include sugars, some amino acids, choline, and organic acids (malic, citric, lactic, and succinic acids). Except for sugars, which serve as a source of energy, the compounds occur in such large quantities that it is illogical to consider them only as intermediates of metabolic pathways. By using nuclear magnetic resonance spectroscopy to study metabolomics, scientists have discovered that these substances occur in specific molar ratios and perform essential functions to maintain the vital activity of a cell or organism as a whole. Therefore, it is feasible to synthesize such NDEs in a laboratory setting. Recently, these solvents have been proposed as potential excipients in pharmaceuticals and drug delivery systems due to their solubilizing properties, diverse viscosities, and intrinsic biological activity [9].

METHODS OF EXTRACTION OF BIOLOGICALLY ACTIVE SUBSTANCES FROM PLANTS

One of the traditional extraction methods is the Soxhlet method, which is relatively time-consuming and uses large volumes of solvent. Advanced methods, in contrast, are characterized by shorter procedure times, low volumes of hazardous organic solvents, ease of operation, high extraction yields, and low energy consumption.

Ultrasonic extraction is a technique used to extract bioactive compounds and obtain total extracts from various plant materials [10]. This method uses low-frequency (>20 kHz) and high-power (80–200 W) energy, coupled with an ultrasonic bath or probe. The underlying principle of extraction is cavitation, which involves the formation of bubbles that disrupt cell walls, thereby facilitating the release of target compounds and the diffusion of solvent into the feedstock. The use of DESs in conjunction

with ultrasound is a more environmentally sustainable approach for the extraction of biologically active compounds than the use of traditional organic solvents [11]. For instance, the ultrasonic extraction method was used to extract biologically active compounds from *Cosmos sulphureus*. Among the various DESs, ratio of choline chloride to lactic acid of 1:2 was the most effective for the extraction of polyphenols, with the optimal extraction conditions being a temperature of 47.5°C, a water content of 32.6%, and an ultrasound power of 4.0 W/mL. The polyphenol content of this method was 21% higher than that of ethanol. The replacement of ethanol with DESs increased the extraction of some individual polyphenols, including chlorogenic acid, kaftaric acid, rutin, and kaempferol, by a range of 10.7%–43.5% [12]. This extraction method has also been applied for the extraction of biologically active substances from mushrooms and fruits [13, 14].

Another method for the extraction of bioactive compounds from plant substrates is microwave-assisted extraction (MAE) [15]. MAE is based on the interaction of electromagnetic radiation waves (usually 2.45 GHz) with the sample through heating and continuous rotation. This process degrades plant cell tissues and the release of active compounds from the intracellular and cell membrane. The effectiveness of this method depends on the nature of the sample and solvent. This extraction method is also cost-effective and requires a minimal amount of solvent, making it a more economically viable option than conventional methods. Additionally, its simplicity of operation makes it an ideal candidate for large-scale industrial applications. In [16], seven main active flavonoids were extracted from currant leaves: trifolin, isoquercetin, rutin, astragaline, quercetin, hyperoside, and kaempferol. Under optimal conditions (temperature 54°C, time 10 min, choline chloride DES as lactic acid in the ratio of 1:2, water content 25%), the extraction process was more efficient than that using traditional solvents. The MAE method using DESs can be employed for the determination of heavy metals in medicinal plant samples [17].

Another promising approach is enzymatic extraction, which typically uses aqueous media, thereby reducing environmental impact [18]. This method relies on the effect of enzymes on the integrity of the plant cell wall, which increases the permeability of the cell membrane, facilitating the efficient extraction of bioactive compounds. In [19], a combined enzyme-ultrasound extraction method was used to extract biologically active substances from tea tree leaves using various NDEs. The efficacy of NDEs was evaluated based on the total content of tannins, flavonoids, and terpenoids. The NDES system with a molar ratio of acetic acid to glycerol of 2:1 exhibited the highest values of tannins and flavonoids, whereas a ratio of acetic acid to glucose of 2:1 yielded the highest

values of terpenoids. Notably, the degree of extraction achieved by enzymatic-ultrasonic extraction using NDESs was significantly higher than that observed in organic solvent extraction and simple ultrasonic extraction with NDESs.

DESs FOR EXTRACTION OF BIOACTIVE COMPOUNDS

Among the most interesting and widely studied plant biologically active substances, the following groups can be distinguished: phenolic compounds, flavonoids, and alkaloids. Each group contains many compounds with unique properties, which makes them promising precursors for pharmaceutical, cosmetic, and food industries (Fig. 2).

This section presents the properties and efficacy of the bioactive compounds most frequently mentioned in the current literature.

Alkaloids

Alkaloids represent approximately 20% of the known secondary metabolites occurring in plants, with about 12,000 compounds having been isolated to date [20]. Therapeutically, alkaloids are well known for their anesthetic, cardioprotective, and anti-inflammatory properties. Additionally, these substances may exhibit antitumor activity [21]. The alkaloids most commonly used in clinical settings include morphine, strychnine, quinine, ephedrine, and nicotine [22]. In [23], three types of bioactive alkaloids were obtained owing to DES extraction of alkaloids from *Berberidis* root. Choline chloride-levulinic acid and betaine-levulinic acid mixtures had a higher extraction capacity than the traditional solvents [24]. In [25], alkaloids obtained by DES lactic acid-glucose-water from *Larrea cuneifolia* exhibited significant antimicrobial activity against *Candida albicans*.

Phenolic compounds

Phenolic compounds derived from plants are well known for their properties and are widely used in various applications, including medicine, food, and cosmetics. Many of these compounds are edible and are found in plants, such as tomatoes, olives, and grapes, or are part of the chemical composition of medicinal herbs, such as mint, lavender, echinacea, and others. The antimicrobial activity and potential applications of phenolic compounds as food preservatives or biocides are extensively discussed in the literature [26]. Phenols, such as chlorogenic, gallic, caffeic, and rosmarinic acids, are secondary metabolites in plants [27]. Due to their antioxidant and anti-inflammatory properties, they can be used as active ingredients in the cosmetic, food, and pharmaceutical industries. Recently, many studies have focused on their medical applications as antitumor agents [28]. Notably, phenols have shown activity in the treatment of neurodegenerative diseases.

In most studies, DESs have been used as an alternative to traditional alcohol-based solvents for the extraction of phenolic compounds [29]. For instance, the extraction of phenolic compounds from *Carthamus tinctorius* uses proline-malic acid and 25% water, and the extraction of phenolic compounds, such as hydroxysaflor yellow A and carthormine compounds was highly efficient [30]. In [31], a variety of DES combinations were used for the extraction of phenolic compounds from olive oil. The synthesis of DESs was based on the use of choline chloride in various ratios with sugars, alcohols, organic acids, and urea. The results of extractions using DESs were compared with those obtained from extractions with an 80% methanol/water mixture. The yields of the two most common secoiridoid derivatives in olive oil, oleacein, and oleocanthal (with pronounced anti-inflammatory and antioxidant properties), increased by 20%–33% and 67.9%–68.3%, respectively, when choline chloride-xylitol

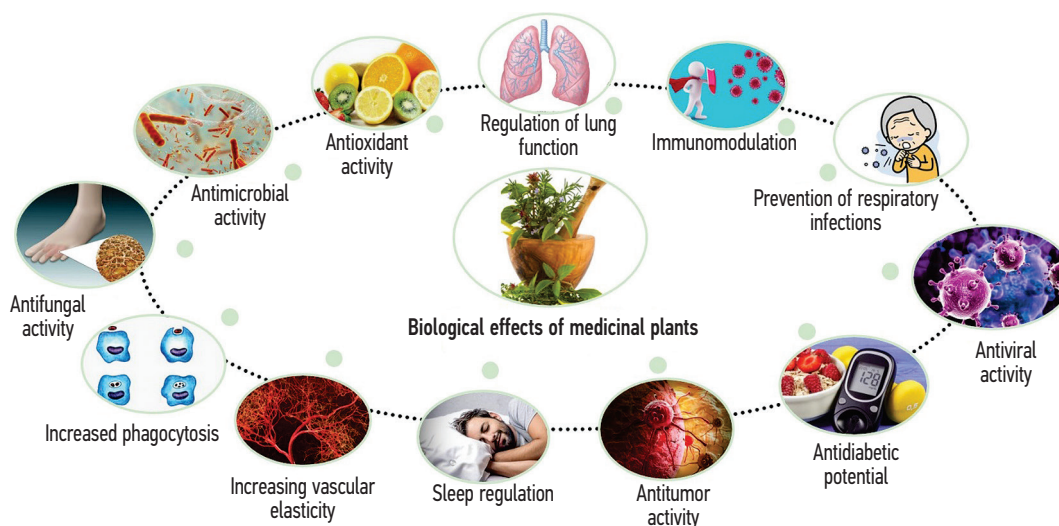


Fig. 2. Some biological properties of plant extracts

Рис. 2. Некоторые биологические свойства экстрактов растений

and choline chloride-1,2-propanediol mixtures were used for extraction.

Flavonoids

Flavonoids are also phenolic compounds, but because of their chemical structure is based on 2-phenylchromene 4-one (flavone), they are considered separately. Like phenols, flavonoids are found in plants all over the world, especially edible plants. The richest source is the fruit of *Prunus* species (e. g., cherries and plums) and *Vaccinium* species (e. g., cranberries and blueberries). Many attempts have been made to use by-products from the forestry and food industries to obtain flavonoids. For example, the use of *Larix decidua* (European larch) bark, a by-product of forest processing [32, 33]. Flavonoids have the potential to serve as precursors of anticancer and anti-inflammatory pharmaceuticals, as well as geroprotective agents in cosmetics.

Recently, DESs have been used as an alternative solvent for flavonoid extraction. This method yields flavonoids at high levels, while also reducing costs and time [34–36]. Additionally, DESs are effective solvents for rutin extraction [36].

Other biologically active compounds

Another class of compounds of great importance are terpenes and their derivatives, known as terpenoids. These compounds occur in many plants and some marine organisms, and many of them have pharmacological activity, including antitumor, antimicrobial, and anti-inflammatory properties [33]. Due to their diversity and interesting properties, they have become important components of food, cosmetics, and pharmaceuticals. The synthesis of such compounds is currently inefficient, which underscores the importance of efficient extraction from natural sources. This is a crucial area of further research and industrial development. Extraction of terpenes and terpenoids from plant substrates can also be performed using DESs [37].

Plant polysaccharides exhibit anticancer, antiviral, and antioxidant activities [38]. In [39], the polysaccharide extract of *Camellia oleifera abel* was obtained using 17 different DESs. The optimal system, which yielded the highest concentration, was a mixture of choline chloride and ethylene glycol with 30% water. This system was more effective than aqueous extraction.

BIOLOGICAL APPLICATION OF PLANT EXTRACTS OBTAINED USING DESS

Antioxidant activity

Several previous studies have used many antioxidant activity assays, including the free radical binding method with DPPH (2,2-diphenyl 1-picrylhydrazyl) and

ABTS (2,2'-azinobis 3-ethylbenzothiazoline 6-sulfonate), iron reducing antioxidant power, and hydroxyl radical scavenging activity (%OH). Some studies have reported a correlation between antioxidant activity and the concentration of polyphenols and flavonoids in medicinal plant extracts [40–42]. The efficacy of these compounds is also significantly influenced by the nature of the extraction solvent. Barbieri et al. [43] reported that extracts prepared with DESs based on organic acids exhibited greater antioxidant activity than extracts obtained by a water-alcohol mixture. Ruta leaf extracts prepared with a eutectic mixture of choline chloride and citric acid in a molar ratio of 2:1 contain the highest amount of phenols and also exhibit high free radical binding activity with DPPH [44]. In another study, Bakirtzi et al. [45] demonstrated that sage extract obtained using lactic acid-choline chloride in a molar ratio of 3:1 exhibit a significantly higher antioxidant capacity than traditional alcoholic extraction methods. This suggests that the use of green solvents can facilitate the extraction of bioactive compounds with high antioxidant properties. Additionally, the high polyphenol content of plants can also result in high antiradical activity. Furthermore, due to the molecular interaction between plant bioactive substances and DESs, such extraction leads to lower oxidative degradation of active substances than that achieved when using conventional methods.

Antibacterial activity

Bacterial growth inhibition is a widely used method in the study of bioactive compounds. Thus, the effect of DES-extracted plant extracts on bacterial growth is extensively studied. For instance, in [46], the bioactive compound phycocyanin was extracted from *Arthrospira platensis* (spirulina algae) using a 1:1 xylose to glycerol system. Phycocyanin is a protein pigment with anti-inflammatory properties, which enhance the functions of the body's immune system. Additionally, it exerts a protective effect against radiation, and has demonstrated high activity against *Escherichia coli* and *Enterobacter aerogenes*.

Other studies investigated the effect of the total content of polyphenols extracted using a mixture of malic acid, glucose, and glycerol (1:1:1) from *Punica granatum* L. The antimicrobial activity of the extract was determined against Gram-negative bacteria. A positive result was observed for *Staphylococcus aureus*, with 90% inhibition observed at a concentration of 0.7 mg/mL⁻¹. These findings confirm the interaction of polyphenols with the cell membrane of microorganisms, leading to microbial cell death or enzyme inhibition [47]. The study also compared the antibacterial activity of extracts obtained by the DES system and the traditional alcohol method. The results demonstrated that the DES system exhibited a higher degree of antibacterial activity.

Antitumor activity

Although the history of antitumor drugs began with nitrogen mustard derivatives and folic acid antimetabolites, plant alkaloids demonstrating antitumor properties are currently being used for the treatment of neoplasms of various localizations. Yew tree alkaloids (taxanes) and periwinkle pink alkaloids (vinca alkaloids) occupy a unique position among cytostatics. However, the most recently registered cytostatics, trabectedin and eribulin, are alkaloids from hydrobionts: the Caribbean invertebrate *Ecteinascidia turbinata* and the marine sponge *Halichondria okadai*, respectively.

Some studies detected the cytotoxic activity of medicinal plants extracted by HER *in vitro* by evaluating cell proliferation. In [48], polyphenolic bioactive compounds were extracted from grape pomace using choline chloride and citric acid (2:1). Cytotoxicity was examined on HeLa (cervical cancer) and MCF 7 (breast cancer) cell lines, with an *in vitro* antiproliferative and cytotoxic activity of 37.61% in the aforementioned cell lines for 72 h. The *in vitro* antiproliferative and cytotoxic activity of the compound extracted from olive pomace using the same DES system was 12.9% for 72 h. Ginsenoside, belonging to the class of triterpene saponins, is a natural product derived from the roots of the Panax ginseng plant. Extraction of ginsenosides from ginseng using a triple DES of glycerol, l-proline, and sucrose (9:4:1) exhibited antitumor activity against human colorectal cancer cell lines at a dose of 58 µg/mL. The DES itself had no cytotoxic effect, as indicated by the MTT test [49].

CONCLUSIONS

DESs represent a promising alternative to traditional solvents for the extraction of biologically active substances from plants. Findings of studies on DES indicate an enhancement in extraction capacity, solubility, stability, biological activity, and bioavailability of bioactive compounds. DESs exhibit high selectivity toward biological targets with minimal toxicity. In the synthesis of eutectic mixtures, the chemical and physical properties of the DES components should be considered to optimize the solubility and stability of bioactive substances. The selection of an appropriate type of DES and the optimization of the extraction process can lead to a high extraction efficiency of plant metabolites. The use of such solvents can assist in the resolution of major challenges in the development of pharmaceutical and nutraceutical formulations.

ADDITIONAL INFORMATION

Authors' contribution. All authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. The contribution of each author: E.V. Andrusenko, R.I. Glushakov, G.A. Redkin — manuscript drafting, writing and pilot data analyses; R.I. Glushakov — paper reconceptualization and general concept discussion.

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