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Application of deep eutectic solvents for separation and determination of bioactive compounds in medicinal plants

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ABSTRACT

The medicinal plants industry, particularly in regard to products rich in biologically active substances for maintaining health, has grown by leaps and bounds in the last decade, with sales of over-the-counter drugs containing these substances growing by billions of dollars. Attention has thus also been paid to the safety and effectiveness of these medicines. We are currently witnessing a rapid increase in the number of publications devoted to the development of new separation procedures that are not only fast and cheap but also more efficient and eco-friendlier, improving both yields and quality of extracts quality without using hazardous organic solvents. The new approaches include those that use deep eutectic solvents (DES), which are characterized by unique parameters. In fact, DESs can be used for both the isolation and determination of biologically active substances in medicinal plants. Therefore, the purpose of the review is to gather details on the application of DESs in the separation of bioactive compounds in medicinal plants and to provide a solid background for future research in this area. To cover these aspects, the available data and references in the field of interest are reviewed and summarized.

1. Introduction

Medicinal plants are a valuable source of a wide variety of chemical molecules having different structures and functionalities that exhibit important biological activities and are linked to a multitude of beneficial properties, such as antimicrobial, anticancer, antiviral, antioxidant and enzyme inhibitory, anti-aging, anti-inflammatory, antihypertensive, neuroprotective and anticoagulant effects (Ali et al., 2019; Lesellier et al., 2021). Medicinal plants are of great importance worldwide, both when used alone and as a supplement to traditional medication (Fig. 1). The remarkable number of reports on the therapeutic properties of medicinal plants combined with long-term experience in folk medicine has led to a growing interest in the use of natural products (Tlili and Sarikurkcu, 2020). With the emergence of antibiotic-resistant bacteria, the study of new bioactive compounds is even more crucial today. According to various sources, medicinal plants serve as the basis for 25–50 % of currently produced drugs used in healthcare (Mahmood et al., 2019; Sinan et al., 2020), and new bioactive compounds from known and exotic plants are being sought worldwide (Fettach et al., 2019). Research in this field is expected to continue for new medicines derived from natural products (de la Luz Cádiz-Gurrea et al., 2021).

Different parts of medicinal plants (leaves, stems, roots, seeds, flowers or fruits) are rich sources of bioactive compounds (Knez Hrnčič et al., 2020). Similar protective and health-promoting effects of plant-derived bioactive compounds have also been found for humans and animals, and their general perception as being safe, natural and having fewer side effects in comparison with chemical drugs makes them highly attractive for consumers and numerous industries. The

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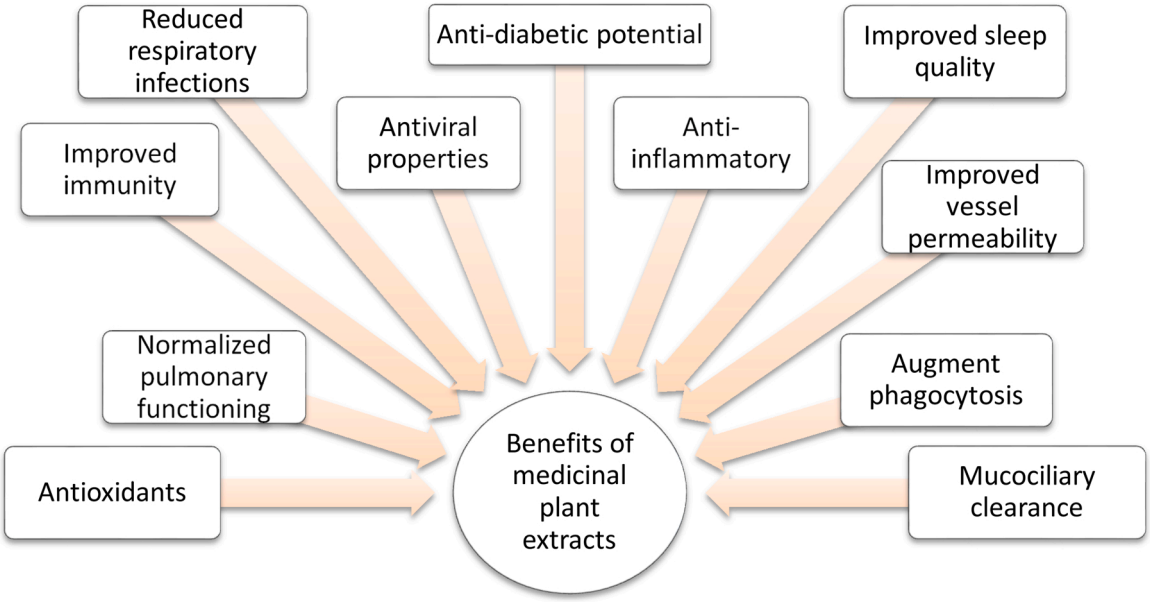

Fig. 1. Schematic representation of the potential beneficial effects of medicinal plant extracts in the prevention and treatment of many diseases.

biologically active compounds in medicinal plants are classified according to various criteria. Following standard pharmacognosy classification, the main chemical compounds present in medicinal plants or their extracts can be divided into several groups based on their structure.

Herbs contain many biologically active substances that may be of interest for the food, pharmaceutical and cosmetic industries. However, in order to use these substances first several tasks need to be solved. In particular, it is necessary (a) to analyze the individual parts of herbs and find out what useful substances they contain; subsequently, (b) procedures for the separation and isolation of the target substances from the herb matrix needs to be developed. However, due to the lack of extraction methods, many bioactive compounds remain hidden from further exploitation. It is therefore necessary to develop eco-friendly methods reducing both energy and solvent consumption. Afterwards, (c) the isolated products need to be analyzed.

In line with current requirements of Green Analytical Chemistry, new alternative solvents (switchable hydrophilicity solvents, SHS (Lasarte-Aragonés et al., 2014), switchable ionic liquids, S-IL (Tang and Ho Row, 2020), and deep eutectic solvents, DES (Tang and Ho Row, 2020; Tang and Row, 2020) have been introduced to analytical chemistry as a replacement for common organic solvents in sample pretreatment step. Deep eutectic solvents is a new class of compounds (Abbott et al., 2004) formed by the mixture of variety hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA). They physical properties are tunable by selection of their individual constituents and HBD:HBA ratio (Rodríguez et al., 2015). However, in general, this eutectic mixture is characterized by simple preparation, low volatilization at high temperature, selectivity, strong dissolving ability and adjustable polarity (Rodríguez et al., 2015). The use of DESs in analytical chemistry for extraction but not only for this has been recently discussed (Shishov et al., 2020; Tang et al., 2021). However, recent studies have presented that potential toxicity and cytotoxicity may be imparted by various DES (Halder and Cordeiro, 2019). In fact, the biological effects of any DES are often found to be significantly different from those of its individual components. The group of DESs called natural deep eutectic solvents (NADES) which are composed of neutral, acidic or basic components that are obtained from nature, have been better evaluated in the range of toxicity and also been tried to replace organic solvents in the fields of separation science, biomedical science, electrochemistry, with excellent biodegradability (Dai et al., 2013).

In this article, we discuss the possibility of using DESs for both the isolation and determination of biologically active substances in medicinal plants. Finally, (d) from a technological point of view, the issue of recovering extracted compounds from their DES extracts must also be resolved using the simplest possible procedure. This aspect is also briefly discussed herein. We hope that this review will inspire many new perspectives and developments in the field of DES applications in the separation of bioactive compounds from medicinal plants.

2. Recently published review papers on the topic

Because the topic of DESs is becoming increasingly attractive for numerous science areas, research and technology, many review articles have been published in addition to the large number of experimental works. The number of such reviews is rather large, so we will mention here only reviews on the topic of our article, i.e. regarding the application of DESs for the analysis of herbs, and with the separation and determination of bioactive substances present in these samples. A few years ago, research papers published from 2013 to early 2017 that were devoted to the DESs application in the area of isolation and enrichment of bioactive substances from natural sources were summarized (Zainal-Abidin et al., 2017). Lately, contributions on the application of DESs in the natural products isolation, mainly from conventional Chinese medicines, have been reviewed. The authors also paid attention to the variables which impacted the extraction efficiency of DESs (Huang et al., 2019). An overview of the application of a deep eutectic solvents in combination with ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE), for the isolation of bioactive compounds from various plants was reported (Ivanović et al., 2020). The DESs application for the isolation of phenolic compounds and main strategies for recovering the selected compounds from the DES after extraction, namely by liquid-liquid extraction using another solvent, solid-liquid extraction applying resin or molecular sieves and precipitation by the addition of antisolvents was discussed (Ruesgas-Ramón et al., 2017).

3. Application of DESs in the analysis of herbal samples

Commonly, water or conventional organic solvents are used to extract biologically active compounds from plant samples. The use of water is limited to water-soluble compounds, while some organic solvents possess hazardous properties (Liu et al., 2019). The use of DESs

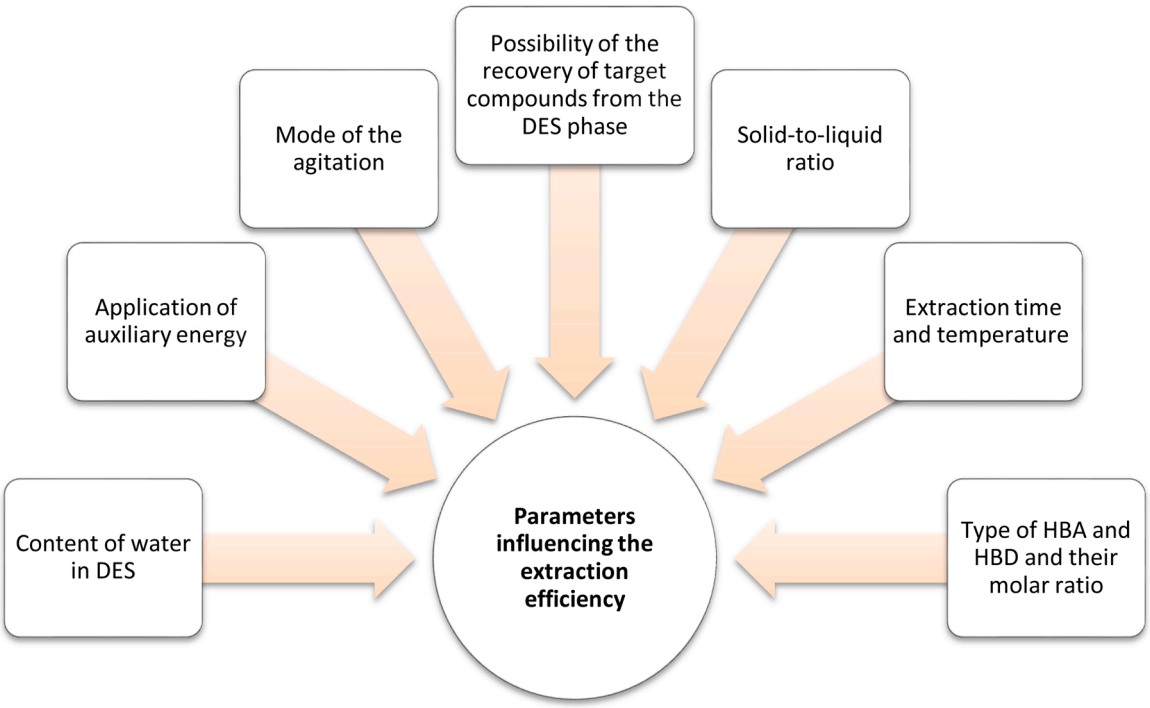

Fig. 2. Schematic representation of DES parameters influencing the extraction efficiency.
DES, Deep eutectic solvent; HBA, Hydrogen bond acceptor; HBD, Hydrogen bond donor.

makes it possible to overcome these disadvantages and limitations. One great advantage of DESs is the possibility of adjusting their properties depending on the need for a specific analytical procedure (type of analyte and sample) by changing the type of HBA or HBD, changing their mutual molar ratio and also by changing the water content (Zainal-Abidin et al., 2017).

3.1. *Effect of variables*

In general, the extraction efficiency of bioactive substances from plant samples is influenced by various parameters that need to be considered when selecting a DES and choosing the extraction conditions. The variables that should be optimized are presented in Fig. 2. Due to the hydrogen bond network between the constituents, DESs are viscous, which negatively affects the extraction efficiency as a result of the limited ability of the target compounds to penetrate into the DES phase. However, a viscosity that is too low is also not favorable to the extraction because it can indicate the decrease of hydrogen bonding between the DES constituents (Liu et al., 2019). Both the addition of water and an increase in temperature lead to a decrease in the viscosity of a DES.

3.1.1. *Effect of water*

The water addition may lead to an important changes in the physicochemical characteristics and the hydrogen bonding network of DESs (Vilková et al., 2020). The properties of a DES can be adapted to the needs of a specific process by adding a suitable amount of water. However, adding water to a DES can result in two opposing effects: on the one hand, (a) decreasing the viscosity (which is beneficial for increasing the extraction efficiency) while also reducing the volume of waste and the final cost. On the other hand, (b) adding a surplus of water weakens the hydrogen-bond network in the DES as well as the interaction between the DES and the analytes, which may ultimately reduce the extraction efficiency (yield) (Liu et al., 2019; Qi et al., 2015; Wang et al., 2018b; Zhao et al., 2019). Recently, El Achkar et al. (2019) provided an overview of the interactions of DESs with water and biological macromolecules (El Achkar et al., 2019).

3.1.2. *Effect of sample agitation and auxiliary energy*

Various methods for agitating samples during extraction have been described, such as stirring, vortexing, shaking, heating and stirring combined, and others. All the above methods have both advantages (increase of high analyte enrichment, lower cost of analysis, the extraction phase which contains target analytes can be easily separated due to the fact that dispersion formed under vortex-mixing is thermodynamically unstable) and disadvantages (poor repeatability, occurrence of extra peaks, peak bordering). Nowadays, to increase the efficiency of extraction, more and more often, procedures based on the so-called auxiliary energies such as ultrasound or microwave radiation are used. In case of ultrasound applications, the extraction time can be shortened. It also allows to reduce the extraction temperature as well as reagent consumption. For the other site, ultrasounds (but also microwaves) can cause changes in the analytes structure (degradation, compositional changes). Application of microwaves also brings many advantages such as faster throughputs, reduction in solvent/reagents consumption, fast on and off switching, selective heating, rapid energy transfer.

3.2. *Target compounds recovery from DES extracts*

For further use of substances separated from plants, their recovery from the DES extracts is necessary. A variety of procedures have been described for this purpose, such as liquid-liquid extraction (Mulia et al., 2019), application of antisolvents (Grudniewska and Popłoński, 2020) and the microporous resin method (He et al., 2019; Liu et al., 2019; Yang et al., 2018). The last-mentioned method is probably the most commonly used.

3.3. *Determination of main bioactive compounds and additional tests*

Before analysis, collected samples are dried by application of controlled temperature and humidity or freeze dried. Furthermore, the dried samples are powdered and sieved. In some cases, additional, auxiliary parameters, such as pH, moisture, proteins, ash, soluble and

insoluble fiber, titratable acidity, total lipids and soluble sugars, are also monitored (Silva et al., 2020). After extraction, the main biologically active substances in the DES extracts are identified and quantified using common analytical techniques, most often high-performance liquid chromatography (HPLC) and gas chromatography (GC) using various detectors. Data on the ability of a DES to stabilize the bioactive compounds in the extract is also important for their further application. Therefore, the influence of some factors, such as heating, light, storage time and ambient conditions in sunlight, on the stability of the compounds in the DES extract is also investigated (Dai et al., 2014).

Further, in order to check the effect of the DES on the features of the extracts, some additional tests are commonly performed. These include the determination of total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and antimicrobial activity.

Total phenolic content is usually evaluated spectrophotometrically using a specific redox reagent (Folin-Ciocalteu reagent). This method allows to determine the total concentration of phenolic hydroxyl groups in the plant extract, and the results are typically represented as gallic acid equivalents (in *mg* of gallic acid per *g* of dried sample) (Alañón et al., 2020; Cao et al., 2018c; Jeong et al., 2018; Wojeicchowski et al., 2021). The basic principle of the aluminum chloride colorimetric method for determination of TFC is based on the fact that aluminum forms acid-stable complexes with the keto- and hydroxyl group of flavones and flavonols. In addition, acid labile complexes can be formed with the specific dihydroxyl groups of flavonoids (Ahmed and Iqbal, 2018). Various standards can be used for the determination of TFC, such as quercetin (Shang et al., 2019), cathechin (Jeong et al., 2018) and rutin (Cao et al., 2018b).

Several assays for evaluating antioxidant activity were reported. The DPPH method is based on the reduction of the reagent (1,1-dipheny-2-picrylhydrazyl, DPPH) in the presence of a hydrogen-donating antioxidant. The stabilization of the free radical DPPH ˙results from the action of antioxidants, which, after the reduction reaction, discolor the methanol solution of DPPH. Quercetin (Shang et al., 2019), Trolox (Barbieri et al., 2020; Jeong et al., 2018; Wojeicchowski et al., 2021), butylated hydroxyl toluene (Liu et al., 2019) and vitamin C (Liu et al., 2019; Peng et al., 2018) were used as the reference antioxidants. Another assay for evaluating antioxidant activity is ABTS, which is based on the ability of an antioxidant to stabilize the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate, ABTS) colored cation radical through the reaction between ABTS and potassium persulfate. The activity was expressed as quercetin (Shang et al., 2019) or Trolox equivalents (Jeong et al., 2018). Ferric reducing antioxidant property (FRAP) was evaluated by the reduction of Fe(III) to Fe(II) in the presence of an antioxidant.

3.4. Analysis of the main bioactive compounds

For the isolation and separation of bioactive substances from plants using DES, the direct solid-liquid extraction approach supported by mixing and heating, or even by ultrasound or microwave radiation, is most often used. Subsequent to the extraction step, further separation of the analytes is achieved by chromatography, mainly using HPLC equipped with an ultraviolet (UV) or mass spectrometric (MS) detector. Examples of the research papers dedicated to DES-based extraction followed by quantification of bioactive compounds in herbal samples are briefly discussed below.

Xia et al. (2015) reported a DES-based procedure to extract rosmarinic acid and salviaflaside from solid *Prunella vulgaris* L. samples. Various types of alcohol-based DESs were prepared and choline chloride–ethylene glycol at a 1:4 M ratio was finally selected. The extracted phenolic compounds were determined using ultra-performance liquid chromatography (UPLC) with the limits of detection ranging from 0.08–0.14 μg mL^{-1}. The alcohol-based DES extraction was compared with previously reported extraction methods, such as ultrasonic extraction with 75 % methanol for 30 min and maceration with 50 % ethanol for 16 h, and the alcohol-based DES extraction showed higher phenolic acid yields (Xia et al., 2015).

Yao et al. (2015) applied polyols-based DES combined with MAE in the extraction of five phenolic compounds from *Pyrola incarnata* Fisch. A series of DES containing the different ratios of choline chloride with different polyols were investigated. The optimal extraction conditions were 30 % water in choline chloride–1,4-butanediol (1:4 M ratio) at 70 °C for 20 min and a liquid-to-solid ratio of 10 mL/g. Microwave-assisted extraction was found to be more effective than heat-stirring extraction and ultrasonic assisted extraction (Yao et al., 2015).

Shi et al. (2020) applied choline chloride–lactic acid (1:2 M ratio) DES for the UAE of saponins, phenylpropionic acid and terpenoids from *Acanthopanax senticosus* (Rupr. et Maxim.) Harms roots. Of the 91 phytochemicals identified, 74 can be extracted with ethanol, and the other 17 were reported for the first time. Therefore, under certain conditions, a DES could replace strong organic solvents for the extraction of ethanol insoluble components (Shi et al., 2020). An efficient DES-UAE procedure for simultaneous determination of five flavonoids for the quality evaluation of *Herba Epimedii* consisting of three herbal species (*Epimedium brevicoum* Maxim., *Epimedium koreanum* Nakai, *Epimedium Wushanense* T.S. Ying) was reported (Guo et al., 2020). The authors synthesized and studied twelve DESs based on various HBDs (polyols, acids and saccharides) and HBAs (choline chloride, L-proline) and selected *L*-proline–ethylene glycol at a 1:4 M ratio with 30 % water content. They reported a reduction in solvent consumption as well as extraction time for icarin compared to the traditional extraction procedure (Guo et al., 2020).

A tissue-smashing extraction (TSE) procedure of four main flavonoids from the seeds of *Oroxylum indicum* Vent. by a DES combined with a UPLC method for their quantification was recently reported (Yin et al., 2020). Various DESs were tested and choline chloride–1,4-butanediol (1:3 M ratio, 40 % water content) was selected and its extraction efficiency was compared with methanol of different concentrations, using TSE. The results presented that the extraction yield of DES was markedly higher than that of methanol. In addition to the extraction yields obtained by TSE being higher than those with UAE, the extraction time of TSE was much less than that of UAE. The possibility of flavonoids recovery from DES extracts was investigated using macroporous resins and a solid-phase extraction (SPE) with C18 column. Due to the cost and convenience of industrial use, macroporous resins appear to be more suitable (Yin et al., 2020).

The application of DESs in various microextraction procedures, including headspace single-drop microextraction (HS-SDME), headspace-solid phase microextraction (HS-SPME), but also hollow fiber liquid-phase microextraction (HF-LPME), have also been reported. Tang et al. developed an HS-SDME procedure for the extraction of bioactive compounds from *Chamaecyparis obtusa* Sieb. et Zucc. leaves using choline chloride with ethylene glycol at different ratios of DESs as the extraction solvents. The DES with a molar ratio of 1:4 showed the best extraction efficiency, using 2 μL of DES, a sample-to-DES ratio of 3:20 g/μL, 30 min extraction time and an extraction temperature of 100 °C. Extracts were analyzed using gas chromatography with flame-ionization detection (GC-FID) without removing the DES from the extracts, with limits of detection at ng/mL levels. The recoveries of the selected terpenoids were calculated and ranged from 79.4 to 103%. The developed HS-SDME procedure was compared with heat-reflux extraction and ultrasonic extraction using methanol (Tang et al., 2014). Jeong et al. reported a one-step sample preparation procedure using a DES as the extraction solvent for chemical characterization of *Mentha piperita* L. (peppermint). Instead of preparing two types of extracts, the peppermint leaves were extracted by a DES composed of choline chloride and D-(+)-glucose at a 5:2 M ratio. The extracted monoterpenes were quantified using HS-SPME coupled with gas chromatography–mass spectrometry (GC–MS), and the same extract was used to assess phenolics (Jeong et al., 2018).

A DES-based HF-LPME to determine traces of selected cinnamic acid derivatives in Chinese medicines and investigation of their rates of

Table 1
Selected examples of the applications of DESs in analysis of herbal bioactive compounds.

Matrix	Analyte	Procedure	Detection	Comments	Ref.
Acanthopanax senticosus root (Rupr. et Maxim.) Harms	Phytochemicals	DES-UAE	UHPLC-ESI-Q-Orbitrap/MS	choline chloride–lactic acid (1:2), 20% water (v/v) solid-to-liquid ratio 1:20	(Shi et al., 2020)
Cajanus cajan (L.) Huth leaves	Phenolics	NADES-MAE	UPLC-UV	choline chloride–maltose (1:2), 20% water liquid-to-solid ratio 30:1 mL/g LOD: <0.15 μg/mL	(Wei et al., 2015a)
Camellia oleifera Abel. flowers	Flavonoids and their aglycones	UAE-DES	HPLC-UV	choline chloride–lactic acid (1:2), 35% (w/w) water solid-to-solvent ratio 40 mg/mL LOD: 0.04–0.07 μg/mL	(Ma et al., 2018)
Chamaecyparis obtusa Sieb. et Zucc. leaves	Bioactive terpenoids	HS-SDME	GC-FID	choline chloride–ethylene glycol (1:4) sample-to-DES ratio 3:20 g/μL LOD: 2.00-3.15 ng/mL	(Tang et al., 2014)
Epimedium pubescens Maxim.	Prenylflavonol glycosides	DES-UAE	HPLC-UV	lactic acid–choline chloride (2:1), 20% water LOD: 0.31–0.66 μg/g	(Wang et al., 2020)
Herba Epimedii (*Epimedium brevicoum* Maxim., *Epimedium koreanum* Nakai, *Epimedium Wushanense* T.S.Ying)	Flavonoids	DES-UAE	HPLC-UV	L-proline–ethylene glycol (1:4), DES:water 7:3 v/v liquid-to-solid ratio 20 mL/g	(Guo et al., 2020)
Ixora javanica (Blume) DC. flowers	Total flavonoids	DES-UAE	UV-Vis	choline chloride–propylene glycol (1:1), 25% water solid-to-liquid ratio 1:25 g/mL	(Oktaviyanti et al., 2020)
Lycium ruthenicum Murr fruit	Anthocyanins	DES-UAE	HPLC-DAD-ESI-MS/MS	choline chloride–1,2-propanediol (1:2), 10% water (v/v) liquid-to-solid ratio 20:1 LOD: 0.036 μg/mL	(Sang et al., 2018)
Olea europaea L. leaf	Phenolic compounds	DES-MAE	HPLC-DAD-ESI-TOF-MS	choline chloride–ethylene glycol (1:2), 43.3% water LOD: 0.03–0.64 μg/mL	(Alañón et al., 2020)
Oroxylum indicum Vent. seeds	Flavonoids	DES-TSE	UPLC-UV	choline chloride–1,4-butanediol (1:3), 40% water solid-to-liquid 20:1 mg/mL LOD: 0.06–0.11 μg/mL	(Yin et al., 2020)
Mentha piperita L. leaves	Volatile monoterpenes and phenolic compounds	DES-UAE followed by HS-SPME	GC-MS, UHPLC-Q-TOF-MS, UV-Vis	choline chloride–D-(+)-glucose (5:2), 25% water sample-to-DES 100 mg/mL	(Jeong et al., 2018)
Typhae sp. pollen	Bioactive flavonoids	DES-UAE	HPLC-UV	choline chloride–1,2-propanediol (1:4), 30% water solid-to-liquid ratio 50:1 mg/mL LOD: 0.05–0.14 μg/mL	(Meng et al., 2018)
Prunella vulgaris L.	Phenolic acids	DES	UPLC-UV	choline chloride–ethylene glycol (1:4), 30-36% vol water liquid-to-solid ratio 12 and 14 mL/g LOD: 0.08–0.14 μg mL^{-1}	(Xia et al., 2015)
Pyrola incarnata Fisch.	Phenolic compounds	DES-MAE	HPLC-UV	choline chloride–1,4-Butanediol (1: 4), 30% water liquid-to-solid ratio 10 mL/g LOD: 0.04–0.14 μg/mL	(Yao et al., 2015)
Salvia miltiorrhiza L. roots	Hydrophilic and hydrophobic components	DES-MAE	HPLC-UV	choline chloride–1,2-propanediol (1:1), 20% vol water solid-to-liquid ratio 0.007 g/mL	(Chen et al., 2016b)
Traditional Chinese medicines	Cinnamic acid derivatives	DES-HF-LPME	HPLC-UV	tetrabutylammonium chloride–hexanoic acid (1:3) LOD: 0.1–0.3 ng/mL	(Zhang et al., 2020)
Eucalyptus and rosemary tea	Cadmium	DES-MNF-LPME	SQT-FAAS	phenol–choline chloride (1:3) LOD: 0.25 ng/mL	(Kasa et al., 2020)

plasma protein binding was recently reported (Zhang et al., 2020). The wall pores and lumen were filled with a hydrophobic DES. Various DESs based on choline chloride, methyltrioctylammonium chloride and tetrabutylammonium chloride (TBAC) as the HBA and ethylene glycol, glycerol, hexanoic acid (HA) and benzyl alcohol as HBD, were investigated. The TBAC–HA DES was selected for detailed study, and various molar ratios from 3:1 to 1:5 were examined. Although an increase in the proportion of HA leads to a decrease in viscosity, at the same time, at a ratio of 1:4, the extraction efficiency also begins to decrease. By comparing the extraction efficiency of the HF-LPME procedure using three different solvents, namely, TBAC–HA 1:3, TBAC–HA 1:1 and HA, the authors showed the advantage of the system using a DES with a molar ratio of 1:3. In addition, they proposed an interesting explanation for both the extraction mechanism of the target active compounds and the determination mechanism of the active compound-plasma protein binding rate using the DES-HF-LPME procedure (Zhang et al., 2020).

The published methods are mainly focused on the determination of organic compounds in medicinal plants, but there are (although only rarely) articles devoted to the use of a DES for the extraction of inorganic components. Kasa et al. (2020) developed a DES-based magnetic nanofluid liquid-phase microextraction method for the preconcentration of cadmium in eucalyptus and rosemary. The magnetic nanofluid liquid phase consists of magnetic metal oxide nanoparticles and a deep eutectic solvent. Choline chloride-phenol was used as the DES. The developed

method demonstrated improvement in detection power compared to the traditional flame atomic absorption spectrometry (FAAS) system (Kasa et al., 2020).

More information on the discussed and other works are summarized in Table 1.

4. Application of a DES for the separation and recovery of bioactive compounds

Deep eutectic solvents have proven to be suitable and very effective solvents for the extraction and separation of biologically important substances from medicinal plants. The separated target substances need to be recovered from the DES solution (Ruesgas-Ramón et al., 2017). Examples of research papers dedicated to DES-based extraction and recovery of bioactive compounds from herbal samples are briefly discussed below.

4.1. Utility of DES as extraction solvents

Dai et al. (2013) tested seven NADESs with different polarity, viscosity, composition and solubilization abilities for the extraction of phenolic compounds of diverse polarity from *Carthamus tinctorius* L. (safflower). Natural deep eutectic solvents were characterized by greater extractability of polar and less polar metabolites in comparison with conventional solvents. The extracted compounds were recovered from NADES with a Diaion resin column (Dai et al., 2013). Later, the same group investigated the stabilization ability of selected NADESs for unstable phenolic compounds (Dai et al., 2014). Several factors which impact on the stability of safflower as well as carthamin extracts were investigated including the effect of heating, storage time, light, ambient conditions in sunlight and content of water in NADES. A stabilizing mechanism was discussed and the authors concluded that the formation of hydrogen bonds or chelation can stabilize the structures of phenolic compounds (Dai et al., 2014). The stability of salvianolic acid B (SAB) from *Salviae miltiorrhizae* L. roots in DESs based on choline chloride with various HBDs (ethylene glycol, 1,2-propanediol, glycerol and 1,4-butanediol) was investigated (Chen et al., 2016a). The degradation products of SAB were analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The stabilizing capacity of a DES is mainly influenced by the water content in the DES; it is less influenced by the structure of the HBD, and the molar ratio of choline chloride to HBD has a minimal effect. A mechanism for improving the stability of SAB in a DES was discussed and the interaction between SAB and the DES was demonstrated with the Fourier-transform infrared spectroscopy (FTIR) spectrum. Finally, choline chloride–glycerol (1:2 M ratio) was chosen as the best solvent (Chen et al., 2016a). Bi et al. (2020) investigated the kinetics of thermal degradation as well as anthocyanins storage stability in *Morus alba* L. (mulberry) and shown that choline chloride–lactic acid DES offers benefits over conventional solvents, implying that DESs can be applied as eco-friendly media for the isolation of bioactive compounds (Bi et al., 2020). Liu et al. (2019) tested three NADESs formed by organic acids and sugars for the extraction of natural pigments called curcuminoids from *Curcuma longa* L. The highest extraction efficiency was obtained in the case of citric acid–glucose DES containing 15 % water (Liu et al., 2019).

4.2. Application of DES as solvents in different extraction techniques

Gao et al. (2020) reported a DES-MAE procedure to extract phenolic compounds from mulberry leaves. Among the 12 tested choline-based DESs, choline chloride–glycerol (1:2 M ratio) with 20 % water content showed the highest extraction efficiency for phenolic compounds. The developed DES-MAE procedure was found to be a more efficient extraction method than heat-reflux extraction and ultrasound-assisted extraction. A macroporous resin was applied to recovery investigated compounds from the DES extracts (Gao et al., 2020). Wei et al. described the isolation and separation of selected flavonoids in *Scutellaria* L. roots by application of NADES-MAE, followed by a direct macroporous resin adsorption as well as desorption process. Choline chloride-lactic acid at a 1:2 M ratio containing 20 % water was selected after the investigation of 13 NADES. Flavonoids were extracted at 60 °C for 12 min using solvent-to-solid ratio of 15:1 mL/g (Wei et al., 2015b). Yu et al. (2021) reported a DES-MAE method for the extraction of iridoids and phenolic acids from *Eucommia ulmoides* Oliv. leaves. They added a small amount of ascorbic acid to modify the acidity of a binary DES consisting of choline chloride and 1,4-butanediol to give a higher extraction yield (Yu et al., 2021).

An interesting approach based on a microwave-assisted natural deep eutectic solvents (MA-NADES) pretreatment procedure coupled with microwave hydrodistillation (MHD) for the extraction of essential oil from *Cuminum cyminum* L. (cumin) seed was reported (Zhao et al., 2019). Natural deep eutectic solvents were used as pretreatment solvents for adsorbing microwaves and dissolving cellulose. The developed procedure was compared with microwave hydrodistillation (MHD) and ultrasound-assisted natural deep eutectic solvent pretreatment combined with microwave hydrodistillation (UA-NADES-MHD). Compared to the MHD and UA-NADES-MHD methods, the proposed method gives a higher yield of essential oil and more identified components.

In another work, ultrasonication-assisted the synthesis of alcohol-based DESs with the application of ultrasonication was investigated. The synthetized products were subsequently examined for the isolation of gingerols from *Zingiber officinale* Roscoe (ginger) powder applying UAE (Hsieh et al., 2020). The DESs were diluted to 75 v/v% before extraction to reduce viscosity. The sieved ginger powder-DES mixture was then extracted in an ultrasonic bath. The concentrations of gingerols extracted by the DES solution were determined using an HPLC-DAD instrument. Some of the prepared DESs showed better extraction efficiency than traditional organic solvents. A field emission scanning electron microscope was used to observe the microstructure of the raw and treated ginger powder. Deep eutectic solvents were shown to disrupt cells and cell walls and also to dissolve weak flakes during extraction (Hsieh et al., 2020). Silva et al. (2020) studied ternary NADES for Vaccinium sp. (blueberry) anthocyanins (Silva et al., 2020). The choline chloride–glycerol–citric acid at a molar ratio 0.5:2:0.5 in the presence of 25 % water was the most efficient for extracting blueberry anthocyanins (Silva et al., 2020). Barbieri et al. studied four choline chloride-based DESs (using choline chloride as the HBA and glycerol, lactic acid, 1, 2-propanodiol and oxalic acid as the HBD, with 10 % w/w of water) in a UAE of phenolic content from *Rosmarinus officinalis* L. Extraction with ethanol was used for comparison. The highest value in the TPC assay was obtained for the choline chloride–1,2-propanediol extract, and a DES formed with choline chloride–glycerol presented the highest value of DPPH (Barbieri et al., 2020).

Qi et al. (2015) suggested a negative pressure cavitation-assisted extraction (NPCE) coupled with macroporous resin enrichment for the isolation, enrichment and separation of selected flavonoids from *Equisetum palustre* L. Nine various DESs were investigated, and choline chloride–betaine hydrochloride–ethylene glycol with a molar ratio of 1:1:2 and 20 % water content showed more effective extraction yields. The direct enrichment of investigated flavonoids in the DES extraction solution were efficiently obtained by application of macroporous resin. The efficiency of the developed DES-NPCE method was compared with 80 % ethanol-based NPCE and DES-UAE. The yields of bioactive flavonoids extraction with the DES were higher than those obtained with 80 % ethanol, and the extraction yields of DES-NPCE were higher than those of DES-UAE (Qi et al., 2015).

The disadvantage of traditional organic solvents is that they often extract only hydrophilic or lipophilic components. Therefore, to increase the extraction efficiency of bioactive components with different polarities, hydrophilic and hydrophobic DESs have been designed. Alsaud et al. (2021) examined 26 types of hydrophilic and hydrophobic DESs for the extraction of β-caryophyllene (Alsaud et al., 2021). The

Table 2
Selected examples of the applications of DESs in the extraction of bioactive compounds from herbal samples.

Matrix	Analyte	Procedure	Comments	Ref.
Artemisia annua L. leaves	Artemisinin	DES-UAE	DES: methyl trioctyl ammonium chloride–1-butanol (1:4) Procedure: solvent-to-solid ratio 17.5:1, ultrasonic power 180 W, temperature 45 °C, extraction time 70 min. Recovery: macroporous resin	(Cao et al., 2017)
Ribes nigrum L.	Anthocyanin compounds	DES-MAE	DES: choline chloride–lactic acid (1:2), 20% (v/v) water Procedure: liquid-to-solid ratio 13:1 mL/g, extraction temperature 45 °C, extraction time 14 min. Recovery: macroporous resin	(Kou et al., 2019)
Vaccinium sp.	Anthocyanins	DES-UAE	DES: choline chloride–glycerol–citric acid (0.5:2:0.5), 25% (v/v) water Procedure: sample-to-solvent ratio 0.1:12 (w/v); ultrasonic bath, 50 min. at room temperature	(Silva et al., 2020)
Carthamus tinctorius L.	Phenolic compounds of diverse polarity	DES extraction	DES: 75% (v/v) proline-malic acid in water; 75% (v/v) sucrose-choline chloride in water; lactic acid-glucose Procedure: solid-to-liquid ratio 30 mg/mL; heating and stirring at 40 °C for 1 h. Recovery: macroporous resin	(Dai et al., 2013)
Cuminum cyminum L. seed	Essential oil	MA-NADES-MHD	DES: choline chloride-L-lactic acid (1:3), 40% (w/w) water Procedure (three-stage extraction): pretreatment stage, fast heating stage, hydrodistillation stage	(Zhao et al., 2019)
Curcuma longa L.	Natural pigments	DES extraction	DES: citric acid-glucose (1:1), 15% (v/v) water Procedure: solid-to-liquid ratio 0.1:10 g/mL, extraction temperature 50 °C with constant stirring for 30 min. Recovery: SPE column	(Liu et al., 2019)
Dioscorea opposita Thunb.	Polysaccharides	DES-UAE	DES: choline chloride-butanediol (1:4), 30% (v/v) water Procedure: liquid-to-solid ratio 30(v/w), extraction temperature 94 °C, extraction time 44.74 min.	(Zhang and Wang, 2017)
Equisetum palustre L.	Bioactive flavonoids	DES-NPCE	DES: choline chloride-betaine hydrochloride-ethylene glycol (1:1:2), 20% (v/v) water Procedure: solvent-to-solid ratio 25:1 mL/g, extraction pressure −0.07 MPa, extraction temperature 60 °C, extraction time 20 min. Recovery: macroporous resin	(Qi et al., 2015)
Eucommia ulmoides Oliv. leaves	Iridoids and phenolic acids	DES-MAE	DES: choline chloride-1,4-butanediol-ascorbic acid (1:1:0.2), 20% water Procedure: 1 g EUL powder, 20 mL DES, 500 W, 20 min. Recovery: macroporous adsorption	(Yu et al., 2021)
Garcinia mangostana L. pericarp	Xanthones	DES extraction	DES: choline chloride–1,2-propanediol (1:3) Procedure: solid-to-liquid ratio 1:10, thermomixer shaking at 500 osc/min. at room temperature for 4 h. Recovery: ethyl acetate/diethyl ether	(Mulia et al., 2019)
Ginkgo biloba L. leaves	Bioactive flavonoids	DES extraction	DES: choline chloride–levulinic acid (1:2), 40% (w/w) water Procedure: solvent-to-solid ratio 10:1 (v/w), stirring at 50 °C and 150 rpm for 15 min. Recovery: macroporous resin	(Yang et al., 2018)
Ginkgo biloba L. leaves	Proanthocyanidin	DES extraction	DES: choline chloride–malonic acid (1:2), 55% (w/w) water Procedure: solvent-to-solid ratio 10.57:1 mL/g, extraction temperature 65 °C, extraction time 53 min. Recovery: macroporous resin	(Cao et al., 2018a)
Ginkgo biloba L. leaves	Various bioactive compounds with different polarities	DES extraction	DES: DES-DES two-phase system (hydrophilic DES–hydrophobic DES) Procedure: solid-to-liquid ratio 1:19.699 g/mL, extraction temperature 65 °C, extraction time 41.954 min.	(Cao et al., 2018b)
Zingiber officinale Roscoe	Gingerols	DES-UAE	DES: Bet–ButG, Lcat–TriG and Lcat–ButG (1:4), 25% (v/v) water Procedure: solvent-to-solid ratio 30:1, ultrasonic bath (300 W, 40 kHz)	(Hsieh et al., 2020)
Gleditsia sinensis Lam. thorns	Flavonoids	DES-UAE	DES: choline chloride–1,4-butanediol (1:4), 20% water Procedure: solid-to-liquid ratio 1:30 mg/mL, extraction temperature 55 °C, extraction time 45 min. Recovery: macroporous resin	(Yu et al., 2020)
Lonicerae japonicae Thunb. Flos	Phenolic acids	DES-MAE	DES: 1,3-butanediol–choline chloride (6:1), 10% water Procedure: liquid-to-solid ratio 9 mL/g, extraction temperature 60 °C, extraction time 20 min. Recovery: macroporous resin	(Peng et al., 2016)
Leptospermum scoparium J. R.Forst. et G. Forst. leaves	β-caryophyllene	DES extraction	DES: menthol–lactic acid (1:2) Procedure: room temperature 25.07 °C, 1000 rpm stirring, 1.09 h	(Alsaud et al., 2021)
Moringa oleifera Lam. leaves	Phytochemicals	DES-UAE	DES: choline chloride–citric acid, 40% water Procedure: solid-to-liquid ratio 1:10 (m/v); sonicator bath 50 °C for 1 h	(Hamany Djande et al., 2018)
Morus alba L. leaves	Phenolic compounds	DES-MAE	DES: choline chloride–glycerol (1:2), 20% water Procedure: liquid-to-solid ratio 20 mL/g, MW temperature 66 °C, extracting time, 18 min. Recovery: macroporous resin	(Gao et al., 2020)
Morus alba L.	Anthocyanins	DES-UAE	DES: choline chloride–lactic acid (1:2), 20% (v/v) water Procedure: solid-to-liquid ratio 10.76 mL/g, temperature 57.24 °C for 31.54 min.	(Bi et al., 2020)
Platycladus orientalis (L.) Franco	Flavonoid glycosides and aglycones	DES-UAE	DES: choline chloride–levulinic acid (1:2), 10% (v/v) water Procedure: solid-to-liquid ratio 25 mg/mL, extraction time 30 min. at 50 °C	(Zhuang et al., 2017)

(*continued on next page*)

Table 2 (*continued*)

Matrix	Analyte	Procedure	Comments	Ref.
Premna fulva Craib	Bioactive flavone di-C-glycosides	DES-UAE	Recovery: macroporous resin DES: choline chloride–1,3-propanediol (1:2), 33% water Procedure: 100 mg sample, 1.4 mL DES:water (1:0.4 (v/v)), sonicated in a water bath at 50 °C for 30 min.	(Chen et al., 2020)
Calamoideae faberii Becc. waste materials	Phenolic compounds	DES-HVE	Recovery: macroporous resin DES: choline chloride–ethylene glycol (1:3), 40% vol water Procedure: solid-to-liquid ratio 1:15, extraction time of homogenate and vacuum-cavitation were 2.0 min. and 25 min., respectively	(Cao et al., 2018c)
Rheum palmatum L.	Anthraquinones	DES-UAE	Recovery: macroporous resin DES: lactic acid–glucose (5:1), 10% water (v/v) Procedure: solvent-to-solid ratio 26 mL/g, extraction time 1.5 h, extraction temperature 82 °C	(Wu et al., 2018)
Rosmarinus officinalis L.	Phenolic compounds	DES extraction	Recovery: macroporous resin DES: choline chloride–1,2-propanediol (1:2), 50 wt% water Procedure: liquid-to-solid ratio 40:1, extraction temperature 65 °C, stirring at 600 rpm for 150 min.	(Wojeicchowski et al., 2021)
Rosmarinus officinalis L.	Phenolic compounds	DES-UAE	DES: choline chloride–1,2-propanediol (2:1), choline chloride–lactic acid (3:1); choline chloride–oxalic acid (1:1), choline chloride–glycerol (2:1), 10% w/w water Procedure: 150 mg sample, 2.85 mL DES; ultrasonic bath for 120 min.; 40 ± 1 °C	(Barbieri et al., 2020)
Salvia miltiorrhiza L.	Bioactive components	DES-UAE	DES: L-proline–lactic acid (1:1), 25% v/v water Procedure: 100 mg sample powder, 1.00 mL extraction solvent, sonicated at 50 °C for 30 min.	(He et al., 2019)
Scutellariae baicalensis Georgi roots	Bioactive flavonoids	DES-MAE	Recovery: ethyl acetate extraction DES: choline chloride–lactic acid (1:2), 20% (v/v) water Procedure: solvent-to-solid ratio 15:1 mL/g, extraction temperature 60 °C, extraction time 12 min.	(Wei et al., 2015b)
Scutellaria baicalensis Georgi	Baicalin	DES-UPE	Recovery: macroporous resin DES: choline chloride–lactic acid (1:1), 40 vol% water Procedure: liquid-to-solid ratio 110 mL/g, extraction pressure 400 MPa, extraction time 4 min.	(Wang et al., 2018a)
Scutellaria baicalensis Georgi	Baicalin	DES-MAE	DES: decanoic acid–tetrabutylammonium chloride (1:2), 33 vol% water Procedure: liquid-to-solid ratio 23 mL/g, extraction temperature 85 °C, extraction time 10 min.	(Wang et al., 2018b)
Sophora japonica L. bud	Rutin (*in vitro antioxidant activity*)	DES extraction	DES: choline chloride–triethylene glycol (1:4), 18.1% water Procedure: liquid-to-solid ratio 10 mg/g, extraction time 28.3 min., extraction temperature 70 °C, stirring at 240 rpm	(Peng et al., 2018)
Humulus lupulus L.	Xanthohumol	DES extraction	DES: choline chloride–propylene glycol (1:2), 5 wt% water Procedure: solid-to-liquid ratio 1:50 (w/w), stirring (500 rpm) at 60 °C for 1 h Recovery: water (as antisolvent)	(Grudniewska and Popłoński, 2020)

DES, Deep eutectic solvent; HVE, Homogenate-assisted vacuum-cavitation; MAE, Microwave-assisted extraction; MA-NADES-MHD, Microwave-assisted natural deep eutectic solvents pretreatment procedure coupled with microwave hydrodistillation; NPCE, Negative pressure cavitation-assisted extraction; UAE, Ultrasound-assisted extraction.

DESs that showed a better affinity for β-caryophyllene are those solvents with hydrophobic characteristics. Deep eutectic solvents with TBAC as the HBA presented higher efficiency of analyte extraction when compared with choline chloride, what can be explained due to differences in solubility in water which affect hydrophobicity (Alsaud et al., 2021). Cao et al. (2018a, b, c) developed a DES-DES two-phase system prepared with two hydrophilic DESs and one hydrophobic DESs at volume ratio of 35:5:40 for extraction of bioactive compounds with different polarities simultaneously from *Ginkgo biloba* L. leaves. The influence of different extraction methods (stirring, air-bath shaking and UAE) on the extraction yields of the two-phase DES system for target compounds was investigated. The stirring method showed the highest extraction yields followed by the air-bath shaking method and the UAE method (Cao et al., 2018b).

Further details regarding these and other works are presented in Table 2.

5. Conclusion and future trends

With increasing consumer demand for greener alternatives that do not contain toxic chemicals and industry concerns about sustainable, non-toxic extraction routes, the use of new extraction technologies in the pharmaceutical industry is being extensively explored. This is also taking place for the isolation and determination of bioactive compounds in medicinal plants. Without a doubt, the pharmaceutical industry in the separation of bioactive compounds sectors, including the extraction sector, should select proper extraction methods but also general procedures that balance product quality, process efficiency, production costs and eco-friendly methods. This is the only way the entire process will be done in accordance with the principles of a sustainable environment. As DESs are being found to be potential extraction media as well as overall separation medium due to their unique properties, it is no surprise that the characteristics of DESs have resulted in a rapid increase in the application of DESs in the extraction of bioactive compounds in medicinal plants. Several important parameters must be considered during an analytical procedure that uses a DES, but the results of such applications bring advantages in the form of increased yields of bioactive compounds as well as its eco-friendly nature. However, further investigations of the use of DESs in the fields of isolation, separation and recovery of bioactive compounds found in medicinal plants are still required. First of all, since DES are still under development, issues related to their production, recovery and re-use require the constant work of scientists. The recyclability of DESs will brings benefits in the range of reduced costs of extraction processes for industrial scale-up. Even more importantly, the development of available extraction protocols for applying DESs, with respect to their performance, the stability of bioactive substances, production costs and their potential effects on human life and health are needed before their use in the pharmaceutical

industries to extract bioactive substances.

CRediT authorship contribution statement

A. Kalyniukova: Conceptualization, Bibliographic research, Writing – Original Draft, Writing – Review & Editing. **J. Holuša:** Writing – Original Draft. **D. Musiolek:** Writing – Original Draft. **J. Sedlakova-Kadukova:** Writing – Original Draft. **J. Płotka-Wasylka:** Writing – Review & Editing, Supervision. **V. Andruch:** Conceptualization, Bibliographic research, Writing – Original Draft, Writing – Review & Editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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