Deep Eutectic Solvents as an Efficient Solvent System Determination the Volatile Compounds with Microextraction

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ABSTRACT: *In the present work, the Headspace-Solid Phase MicroExtraction (HS-SDME) process was established utilizing a novel version of Deep-Eutectic-Solvents (DESs) for extracting and preconcentrating essential oils in plants followed by GC-FID determination. DESs seem to be a cost-effective and attractive alternative for the usage of ionic solvents in biotransformation. DESs are usually developed through gentle warming and stirring of two (low-cost and bio-based) salts. DESs have several superiorities over ionic liquids such as their ease of preparation, low production cost, and permit for large-scale applications. HS-SDME was made in the current research in order to extract volatile compounds by the use of Deep Eutectic Solvents (DESs) serving as extraction solvents. HS-SDME was constructed as a solvent-minimized extraction method. However, there are a rare number of studies investigating the Deep Eutectic Solvents (DESs) applications to the HS-SDME of bioactive compounds. Deep eutectic solvents, created by mixing choline chloride (ChCl) and p-Chlorophenol at varying ratios were utilized for extracting essential oils from Echinophora platyloba DC via HS-SDME in the present study. Afterward, headspace single-drop micro-extraction (HS–SDME) was conducted, being connected to gas chromatography. HS-SDME is a quick and simple method in comparison with heat reflux extraction. In addition, it is possible to use DESs in HS-SDME for extracting various volatile compounds.*

KEYWORDS: *Deep eutectic solvent; Echinophora platyloba DC; Gas chromatography-mass spectrometry; single-drop microextraction.*

INTRODUCTION

Echinophora genus belongs to the Umbelliferae family and contains ten various species with yellow or white flowers. *Echinophora platyloba* DC is a major traditional plant having a long history in Iranian traditional medicine. *Echinophora platyloba* is generally called “Khusharizeh”, “Khosharuz”, “Tigh-Turagh”, “Tigh Masti”, “Koshandar”, “Kouzang”, “Tologh-Oti”, and “Tanghezand” in folklore. It is a spiny plant that has cylindrical fruits and yellow flowers on a single stem. In sandy soil types, it grows at 1400 to 2000 m above the sea level from September to October and it then starts its sleeping period. Its growing season is changed by the temperature and altitude change. In Iranian traditional medicine, *Echinophora platyloba* is utilized as a folkloric medicinal herb, and it is used

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as a food flavoring in dairy products like yogurt and cheese. In the southwestern regions of Iran, it is used primarily as an antifungal preservative in order to prevent the fungal growth on some foods that are traditionally made, such as pickled cucumber and tomato paste [1-2].

The studies have broadly evaluated Ionic Liquids (ILs) in biotransformation with various intentions, for instance, performance additives, IL membrane-based processes, nonconventional (co-)solvents, and coating agents for stabilizing/immobilizing enzymes. Academic research has labeled them as 'green solvents'. Nevertheless, environmental dimensions associated with ILs have been seriously addressed recently, which states that many ILs that are mainly used cannot be considered as 'green derivatives'. Similarly, because of the high costs of the ILs they cannot be practically used. The third generation of ILs is under construction, which attempts to combine sustainability and the auspicious benefits of ILs. Similarly, deep-eutectic-solvents (DESs) seem to be a cost-effective and attractive alternative for the usage of ionic solvents in biotransformation. DESs are usually developed through gentle warming and stirring of two (low-cost and bio-based) salts (such as urea and choline chloride). Primary successful applications of DES in biotransformations have been recently indicated. It can be anticipated that knowledge in biotransformations and (second generation) ILs could be realized as actual applications through the usage of these DESs, and third-generation ILs, in the future [3].

Ionic liquids (ILs) (low-temperature molten salts) are combinations of anions and cations, which are not compacted well among them. Thus, they stay liquid at low-to-mild temperatures. A combination of bulky asymmetric cations with anions with weak coordination often leads to low melting points. The interest in ILs is due to the chance of modulation of physicochemical characteristics *via* the selection of combinations of anions and cations. For example, ionic liquids can be designed that are immiscible with high polarity or low polarity organic solvents, or both of them. DES is only the mixing of a hydrogen-bond donor and a solid salt in varying proportions. The combination of urea (m.p. 1328 ºC) and choline chloride (m.p. 3028ºC) is a classic example, which forms a DES with a 128ºC melting point. Although it is not evident that DES can be described as a suitable IL as some of the structures (like urea) are not charged, there is this belief that we can extrapolate many characteristics and added values of ILs to DES [4].

Shemirani et al. originally introduced deep eutectic solvent-based magnetic bucky gel (DES-MBG) as a novel kind of nanofluid with magnetic susceptibility features [5]. *Abbott et al.* developed Deep Eutectic Solvents (DESs) [6]. DESs are novel environment-friendly solvents that are achieved through a combination of a Hydrogen Bond Donor (HBD) (for example, glycerol, urea, carboxylic acids, sugars) and Hydrogen Bond Acceptor (HBA) (including, ChCl salt). Hydrogen bond interactions make an association between HBA and HBD [7-9]. Low cost, simple synthesis process, no need for further purification steps, acceptable pharmaceutical toxicity, and biodegradability are among the favorable properties of DESs. Room-Temperature Ionic Liquids (RTILs) contain ions with a negative and positive charge, which are available as liquids at low temperatures (<100 C). The Ionic Liquids (ILs) have low melting points, which is due to the combination of bulky asymmetric anions or cations that reduce the lattice energy. Subsequently, the melting point of the resulting ionic medium is lowered. Unlike Volatile Organic Compounds (VOCs), ionic liquids have various salient characteristics, including immiscibility with many VOCs, acceptable extractability and solubility for different inorganic and organic and organometallic materials, high electrical conductivity, adjustable physical properties, and chemical structures, improved thermal stability, broad electrochemical windows, insignificant vapor pressure, great design, different solvation interactions, and intrinsic conductivity [10]. Recently, ILs have been emerging as eco-friendly and cost-effective solvents for dissolving biopolymer matrices (hemicellulose, protein, cellulose, etc.) that have also been highly efficient [11,12]. With this feature, better accessibility to essential oil would be provided by eliminating subcellular and cellular structures in herbal materials. In addition, the direct distillation of essential oil can be facilitated by the thermal stability and nonvolatility of ILs [14]. IL-assisted microwave distillation with headspace single-drop microextraction followed by GC–MS was presented by *Fu et al.* for the purpose of quick identification of EO constituents in F. forsythia [15]. In this study, an SDME process was set using a new version of DESs for pre-concentrating and extracting essential oils in herbs followed by GC-FID determination. The different DESs were used in the extraction liquid phase. Variables, such as drop volume, the weight of the plant, extraction time, and temperature were optimized

and the optimized procedure was applied to determine the volatile compounds of medicinal plants.

EXPERIMENTAL SECTION

Reagents and materials

All terpenes, solvents, and reagents were prepared by Merck, Sigma-Aldrich, or Fluka, and no further purification was done. Choline chloride, phenol, ethylene glycol (purity≥0.99, and analytical-grade) sodium chloride, p-Chlorophenol (Purity≥ 99%), sodium hydroxide, and hydrochloric acid were obtained from Merck. The aerial parts of *E. platyloba* were collected in the flowering period from the Northwestern area of Iran; in July 2019 and a voucher specimen (2421 ARH) was placed in the Herbarium of the Faculty of chemistry, Kermanshah, Iran. The plant materials were dried in the air, and they were kept in sealed bags in the cool.

DES Preparation

For preparing deep eutectic solvent, 0.13 g ChCl as a hydrogen bond acceptor and the appropriate amount of different hydrogen bond donors like 4-chlorophenol, phenol, and ethylene glycol were mixed in a one mL screw cap tube. Then, the cap was closed, and the tube was put in a water bath for ten minutes at 85 ºC. It was then vortexed for five minutes. This heating/vortexing cycle was reiterated one more time so that a homogeneous liquid was achieved.

Isolation of essential oil

The aerial sections of *Echinophora platyloba* DC. (50 g) that had been dried at the air were ground, and they were hydrodistillated for three hours by the use of a Clevenger-type apparatus as advised by British Pharmacopeia. Shortly, the plant was placed in water and warmed to boiling. Then, the essential oil and water vapor were evaporated and ultimately gathered in a condenser. The anhydrous sodium sulfate was used for isolating and drying the distillate. The oil was kept at 4 ◦C until the time of analysis by GC–MS and GC.

Instrumentation

An FID detector system and a Hewlett-Packard HP 7890 series GC armed with a split/splitless injector were utilized for determination. Helium (99.999%) was used as a carrier gas with 1.1 mL/min flow rate. The column was kept for 2 min at 100 ºC and incremented to 200 ºC at a rate of 20 ºC/min. Then it was elevated to 280 ºC at 10 ºC/min and maintained for 10 min at this temperature. The temperature of the injector was adjusted at 300 ºC, and all injections were performed in a pulse spiritless state (sampling time of 10s).

The headspace single-drop microextraction (HS-SDME) procedure

The general headspace SDME process that was used the current study was based on our previous guidelines [15, 16]. A 25mL round-bottom flask with 3g of the the dried plant was warmed at 85 ◦C using a mantle. The Hamilton syringe was washed and primed seven times with the solvent/standard solution. The 5µL micro syringe having the suitable DES was fastened above the vial of the sample solution. Prior to the extractions, the syringe was washed 10–15 times with the deep eutectic solvent so that air bubbles are not formed and the carryover of compounds between extractions is prevented. Then, the microsyringe was lowered and its needle passed through the vial septum until the needle tip was 5–15mm above the stirred sample solution's surface (that depends on the volume of the sample). Afterward, the syringe plunger was pushed down for suspending the solvent drop from the tip of the needle. The drop was exposed to the head-space of the stirring sample solution in order to pre-set extraction time. Following extraction, the plunger was removed, and the micro drop was retracted back into the syringe and injected into the GC injection port directly.

RESULTS AND DISCUSSION

A simple approach was applied for optimizing the parameters affecting extraction efficiency. Using a simple approach can cause a significant reduction in the number of experiments needed for gaining maximal extraction efficiency. During optimization, the relative areas of four main peaks in the GC-MS chromatogram were checked. In the simple approach, (n + 1) primary experiments were designed (n denotes the number of parameters affecting extraction efficiency in SDME technique), the conditions for the worst response were mirrored, and the reflection procedure was reiterated until no more improvement was seen in the response. Some of the reflections were adjusted if necessary. Table 1 indicates the conditions for the initial experiments and the experiments subsequently designed

Table 1: Preparation of DESs.

HBA(ChCl) amount (g)	HBD	Mole ratio of HBD : HBA	HBD Amount (g)	DESsynthesis
1.39	ethylene glycol	0.5:1	0.31	NO
1.39		1:1	0.62	NO
1.39		1.5:1	0.93	NO
1.39		2:1	1.24	Yes
1.39	phenol	0.5:1	0.47	NO
1.39		1:1	0.94	NO
1.39		1.5:1	1.41	NO
1.39		2:1	1.88	Yes
1.39	4-Chlorophenol	0.5:1	0.64	NO
1.39		1:1	1.28	NO
1.39		1.5:1	1.92	NO
1.39		2:1	2.56	Yes

for DES-HS-SDME. The experimental conditions were achieved using a modified reflection approach. The modifications were usually done according to the practical constraints of some factors like micro drop volume (manipulation of larger drops is difficult). According to the results, there are positive impacts of sample weight, extraction time, and micro drop volume on the extraction efficiency of DES-HS-SDME approach.

SDME optimization

In order to obtain high extraction efficiency of the analytes using DES-HS-SDME for extracting essential oils from *E. platyloba*, different parameters on the amounts of extracted essential oils were investigated. So, to develop SDME methodology for the determination of essential oils by DESs, several parameters control optimum performance, such as the selection of the type of extraction phase and optimization of sampling time and temperature, and DESs volume.

Synthesis and select the type of DES

Different DESs including ChCl as an HBA and 4-chlorophenol, phenol, and ethylene glycol as the HBDs at different mole ratios were prepared. In all cases, the mixtures were added into 10 mL screw cap test tubes and they were capped and placed into a water bath at 75 C for 10 min. Then they were vortexes for 5 min. The heating/vortexing cycle was repeated another time. Only in the case of the 1: 2-mole ratio of ChCl and the phenolic compounds were the homogeneous liquids (DESs) obtained [17-19]. The results were shown in Table 1.

Extraction with the different DES type

Before optimization of the extraction parameters, the effect of the DES type on the extraction of the collected analytes in the GC was investigated. The choice of an appropriate solvent is essential for the SDME method, which depends on the extraction of the target compounds in this medium. Because of the critical effect of the solvent on DES-HD–SDME, three different DESs including ChCl as a HBA and 4-chlorophenol, phenol, and ethylene glycol as the HBDs were prepared and evaluated. The peak heights obtained for four major components are compared in Fig.1.

The most intense signals and the least overlap were obtained by use of ChCl: 4-chlorophenol deep eutectic solvent. Fig. 1 shows that the DES produced from ChCl: 4-chlorophenol is the most effective extraction solvent and gives the highest peak areas and extraction efficiencies for the selected analytes among the tested DESs. Therefore, it was selected as the solvent for further experiments.

DES volume

The micro drop volume impact on the extraction efficiency for the target analytes was examined. It is clear that using a large organic drop leads to increased analytical

Table 2: Experimental conditions used and results obtained for the DES-SDME experiments performed in the simplex optimization procedure.

Exp. no	Sample weight (g)	Extraction time (min)	Temperature (°C)	Droplet volume(µl)
1	2	30	70	1
2	3	40	70	1
3	3	30	90	1
4	3	30	70	1
5	3	30	70	2
6 (Refl.[a])	3	35	80	1.5
7 (Refl.)	3 333	38	85	2
8 (Refl.)	3	27	92	2
9 (Refl.)	3	35	73.5	2
10 (Refl.)	3	30	80	2

a) Reflection

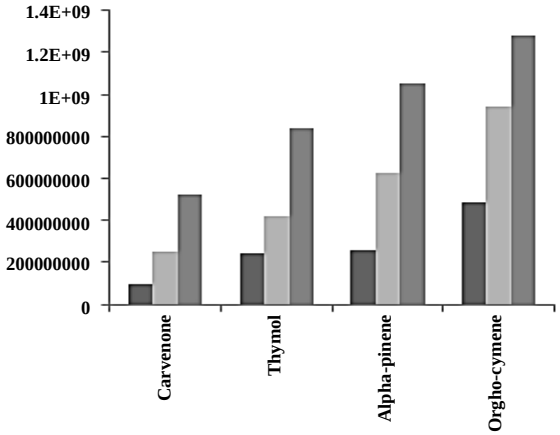

Fig. 1: Effect of solvents on extraction efficiency.

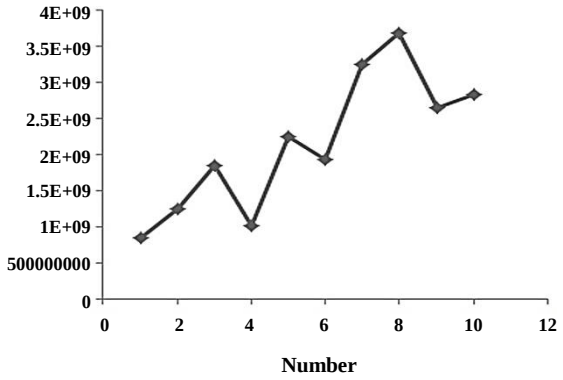

Fig. 2: The response (sum area of four main peaks) for the designed experiments are mentioned in Table 2.

response. Nevertheless, the operation of larger drops is difficult and they are not reliable. Moreover, the larger injection volumes lead to band widening in capillary GC. Hence, a micro drop volume of 2 µL was used since it assured that a reproducible and stable micro drop is formed, although there was some penalty in the form of sensitivity loss.

Sample temperature

The extraction temperature has a dual impact on headspace SDME. The vapor pressure of analytes would be higher as a result of increasing temperatures. Thus, concentrations of analytes is increased in the headspace. Nevertheless, the partition coefficients of analytes in extraction solvent and headspace would be decreased by the temperature. With applying a hanging drop for half an hour in the headspace, the temperature impact was studied in the range of 60–92 ºC. The extraction ability was enhanced with the increase in the temperature over 90 ºC, as a result of increasing the distribution constant of analytes between the aqueous phase and headspace. However, a small reduction was observed in adsorption capacity in most compounds by increasing the temperature over 90 ºC. It can be attributed to decreased partition coefficients of analytes in the headspace and hanging drop as adsorption is an exothermic process. Hence, 90 ºC was selected as the optimal temperature. The advantages were shown in Fig. 2 and Table 2.

Table 3: Constituents of the oil of Echinophora platyloba DC.

Compounds	RI[a]	HD[b] Area%	DES[c] Area%	RSD[d]
α-Thujene	930	0.38	0.45	5.6
α-Pinene	937	6.23	3.80	7.3
Camphene	948	0.52	0.21	9.1
Sabinene	975	0.72	0.32	5.9
β- Pinene	973	0.10	0.05	3.9
Myrcene	990	1.06	0.56	6.3
1-Decene	991	0.17	0.03	7.1
α- Phellandrene	1005	30.65	20.26	14.0
ortho- Cymene	1025	8.20	5.65	9.4
β- Phellanderne	1027	0.32	0.03	13.0
Z-beta- Ocimene	1041	1.34	1.02	5.8
E-β- Ocimene	1052	29.06	17.92	8.6
γ- Terpinene	1060	0.32	-	4.6
Terpinolene	1073	0.30	-	5.9
α-pinene oxide	1095	0.11	-	5.9
Trans-sabinen hidrate	1097	0.08	-	7.6
Linalool	1098	1.50	-	10.8
Limonene oxide -trans	1139	0.21	-	6.0
cis- Verbenol	1142	0.12	-	10.0
Thujanol-neo-3	1149	0.19	-	9.9
Menthone	1154	0.18	0.08	7.9
Trans-beta-terpineol	1160	0.14	0.02	12.1
Terpin-4-ol	1173	0.24	0.03	9.0
Carveol	1218	0.76	0.04	8.3
Carvone	1241	0.14	0.08	18.1
Carvenone	1248	2.97	1.86	10.5
Geraniol	1256	0.21	0.01	9.0
Thymol	1287	4.31	2.24	8.3
Carvacrol-ethyl ether	1296	0.27	0.52	9.0
Terpin-4-ol-acetate	1330	0.21	0.06	7.0
Bicyclogermacrene	1492	0.44	0.03	6.6
α-(E,E)-franesene	1505	0.08	0.02	9.4
Germacrene B	1552	0.26	0.01	10.2
Spathulenol	1576	0.14	0.07	8.6
Cedrol-epi	1609	0.06	0.02	9.5

a) Retention indices using a HP-5 column. b) Relative area (peak area relative to total peak area) for hydrodistillationmethod.
c) Relative area (peak area relative to total peak area except for the solvent peak) DES method. D) RSD values for DES method (relative peak area).

Extraction time

HS-SDME is an equilibrium-based approach, and there is a direct association between the extracted amount and extraction time. Thus, extracting time is a critical factor that influences the method's efficiency. Extraction was implemented at 27-38 minutes to determine the impact of extraction time on the effectiveness of the method. Fig. 2 indicates the findings, representing the peak area against

extraction time profiles. As expected, at first, with increasing the extracting time, the extraction efficiency increased. Following about 25 minutes, equilibrium was achieved for all analytes under study. According to these findings, we selected a period of 27 min for the experiments. Table 2 shows results for a comparison of the two approaches. For microextraction of the solvent, and expensive and dedicated device is not necessary. 27 compounds were separated and determined in *E. platyloba DC* by the use of DES-SDME followed by GC–MS, which supported the results obtained from the hydrodistillation technique. In comparison with hydrodistillation, the advantages of DES-SDME include time-saving, simplicity, and the need for a small amount of sample. Relative SD values were calculated by relative contents of all identified components and were utilized for evaluating the precision of the method. According to Table 3, the RSD values obtained for all essential oil compounds were between 3.9-14.0%.

CONCLUSIONS

In the present work, an SDME process was established utilizing a novel version of DESs as the efficient microextraction for extracting and preconcentrating some essential oils in plants followed by GC-FID determination. A simple approach was applied for optimizing the parameters affecting extraction efficiency. DESs as a novel candidate of solvents involves the advantages including non-flammability, non-volatility, low vapor pressure, good thermal stability, extensive liquid range, good biodegradability, low toxicity, and capability for being reutilized. Before optimization of the extraction parameters, the effect of the DES type on the extraction of the collected analytes in the GC was investigated. The suggested process is very rapid and simple. The total analysis time (specimen pre-treatment plus chromatography) is around 30 min. For microextraction of the solvent, a dedicated and expensive device is not required. Using DES-SDME followed by GC–MS, 27 compounds were separated and identified in *E. platyloba DC*, which confirmed the obtained results of the hydrodistillation method. Compared with hydrodistillation, DES-SDME provides the advantages of a small amount of sample, time-saving, and simplicity.

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