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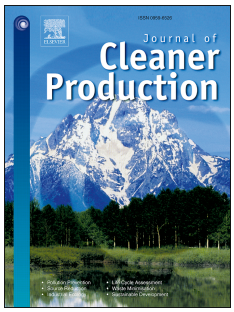

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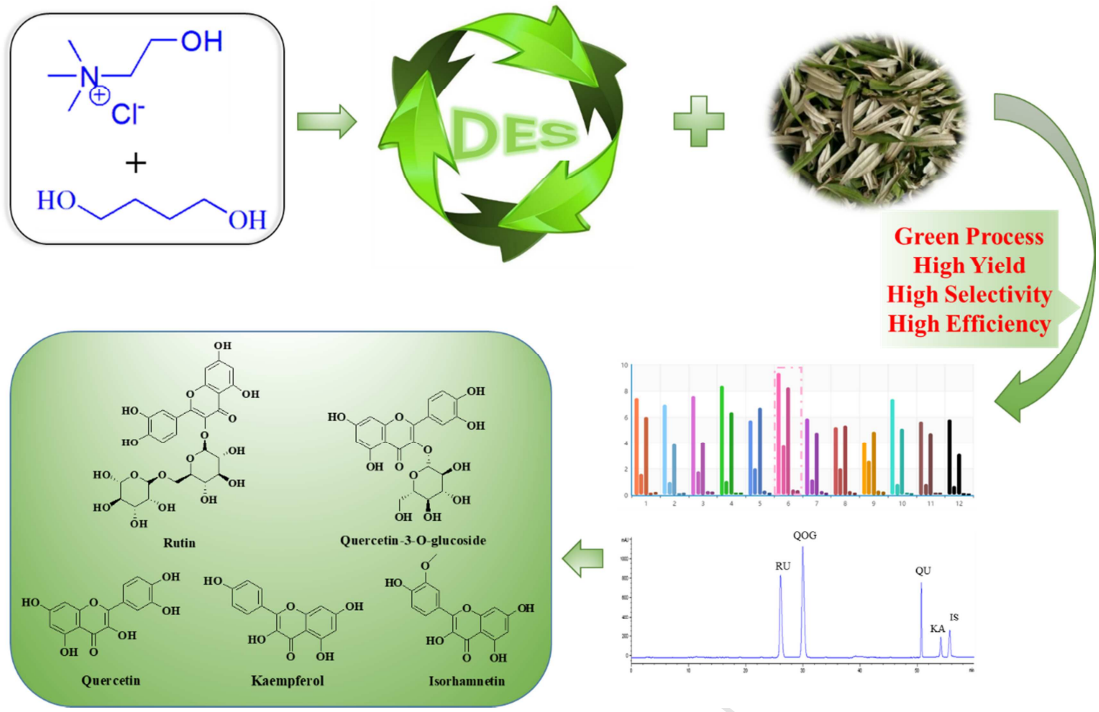
Graphical Abstract



Sustainable deep eutectic solvents preparation and their efficiency in extraction and enrichment of main bioactive flavonoids from sea buckthorn leaves

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Abstract:

Deep eutectic solvents (DESs) have naturally emerged as an alternative for traditional organic solvents for their efficiency on extracting natural products from plant materials. In the present study, 12 kinds of DESs (choline chloride as the hydrogen bond acceptor) were firstly prepared and applied to extract five main flavonoids (namely rutin, quercetin-3-O-glucoside, quercetin, kaempferol and isorhamnetin) from sea buckthorn leaves (SBL) combined with microwave-assisted extraction. The results showed that the extraction efficiencies of target flavonoids by DES were significantly superior to 70% ethanol. Aiming at obtaining the optimal procedure, the extraction conditions were statistically investigated. Under the optimal conditions in pilot-scale application, the total maximum extraction yields of five main flavonoids reached to 20.820 mg/g, which was 1.321-2.400 folds to those by the traditional extraction methods. Meanwhile, the following enrichment of targets compounds from DES extraction solution exhibited excellent recoveries in the range of 72.36-84.99% were achieved by macroporous resin AB-8. In addition, the enriched flavonoids by AB-8 resin exhibited better antioxidant activities as compared with those unenriched. The results indicated that the potential of the developed method as a promising safe and sustainable alternative for selectively extraction and separation of bioactive substances from plant materials.

Abbreviations

SBL, sea buckthorn leaves; RU, rutin; QOG, quercetin-3-O-glucoside; QU, quercetin; KA, kaempferol; IS, isorhamnetin; ILs, ionic liquids; DES, deep eutectic solvent; HBD, hydrogen bond donor; HBA, hydrogen bond acceptor; ChCl, choline chloride; MAE, microwave-assisted extraction; DES-MAE, deep eutectic solvent-based microwave-assisted extraction; HRE, heat refluxing extraction; UAE, ultrasound-assisted extraction; DW, dry weight; BV/h, bed volume per hour.

Keywords: Deep eutectic solvent; Microwave-assisted extraction; sea buckthorn leaves; Flavonoids; Macroporous resin; Antioxidant activity

1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L.), a thorny nitrogen-fixing deciduous shrub that is native to Eurasia and is now widely distributed in various regions of the world (Périno-Issartier et al., 2011). It is reported that sea buckthorn berries contain a remarkable quantity of nutrients and bioactive substances, such as flavonoids, carotenoids, tocopherols, sterols, lipids, vitamins, tannins, etc., which are contributed to its nutritional value and the potential health effects (Sharma et al., 2008; Kallio et al., 2002; Xu et al., 2011). Sea buckthorn leaves (SBL), as an inexpensive and unexploited byproduct, are rich in polyphenolic compounds, but fewer studies have been published as compared to berries and seeds (Michel et al., 2012). Flavonoids are the most commonly polyphenolic compounds found in food, and approximately 15,000 flavonoids have been separated and identified from plants (Ma et al., 2016). Rutin, quercetin-3-O-glucoside, quercetin, kaempferol and isorhamnetin are five major flavonoid compounds in the SBL extracts, which possess stronger antioxidant activities since they have a relatively low oxidation potential 3-hydroxyl group, which can be oxidized irreversibly thus avoiding redox cycling (Hendrickson et al., 1994; Guo et al., 2017). It is indicated that the SBL extract as well as isolated compounds possess pharmacological and therapeutic activities including antioxidant, cytoprotective, immunomodulatory, cardioprotective, anti-inflammatory and a significant wound-healing activity, SBL has been used as traditional herbal medicine to treat diseases in some countries (Geetha et al., 2002; Upadhyay et al., 2010; Ganju et al., 2005; Suryakumar and Gupta, 2011; Xie et al., 2015). Considering SBL nutritional and medicinal potential, SBL extracts, tea and tea powder, are main products since they are important ally of human health (Mathew et al., 2011). However, the normal extraction process from SBL is traditional extraction procedure with alcohol solvent, some questions such as lower extraction yields, lower contents of active constituents in the extracts, higher energy consumption, and lower environmental-friendliness. Therefore, it is urgent to develop highly efficient extraction procedure for SBL extraction including green extraction solvent and mass-transfer technique.

Nowadays, the demand of green chemistry epoch for solvents used in industries such as food, pharmaceutical and cosmetic is increasingly stringent, the green, eco-friendly and sustainable alternative solvents to substitute for hazardous and toxic ones are required. To improve the

protection of human health and the environment from the risks associated with the use of hazardous organic volatile solvents, tremendous efforts have been devoted for the development of alternative green reaction media (Khandelwal et al., 2016). Recently, ionic liquids (ILs) have been regarded as the "perfect alternative green solvents" reported by a number of literatures due to the outstanding superiorities of negligible volatility, biocompatibility and high solubility, etc. (Ho et al., 2014). ILs have been applied in different areas such as extraction solvent and catalyst in chemical or enzymatic reactions (Ana et al., 2013). Nevertheless, the greenness of ILs is challenged by their poor biodegradability, biocompatibility and relatively high solubility in water as pollutants, especially the potential environmental hazards and high costs (Plechkova and Seddon, 2008, Romero et al., 2008). These drawbacks hamper the industrial application of ILs. Meanwhile, a novel class of sustainable solvent named natural deep eutectic solvent (NADES) based on natural compounds including carboxylic acids, polyols and sugars has naturally emerged as alternative to ILs and organic solvents used by academia. DES is generally composed of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA), and the formation of DES results from strong hydrogen bond interaction between HBD and HBA. Choline chloride (ChCl) is one of the most widespread HBAs applied for the synthesis of DES due to its superiority of being inexpensive and readily available. The physical characters of DES are similar to ILs including viscosity, density, surface tension, and conductivity (Zhang et al., 2012). Besides, DES possess more outstanding characteristics compared with ILs, which enables the DES to be a substitute for IL and embrace the Principles of Green Chemistry. Due to the excellent properties, DES has been widely applied in the fields of electrochemistry, organic synthesis, biocatalysis, biodiesel preparation, metal-catalyzed or metal-mediated organic reactions and extractions (Smith et al., 2014; Vidal et al., 2016, Xing et al., 2018, García-Álvarez., 2015, Li et al., 2016; Aroso et al., 2017). Considering the special effects of DES on dissolving both polar and nonpolar metabolites (Paiva et al., 2014), it is rather interesting to investigate the extraction efficiencies of tailored-DESs.

In the present work, twelve DESs based on choline chloride and natural, renewable products was rationally designed and prepared (Fig. 1). Moreover, a microwave-assisted-DES extraction technique was developed for rapid and efficient extraction of five target bioactive flavonoids from SBL, which has not been reported yet up to our knowledge. The selection of DES and microwave-assisted extraction (MAE) parameters

were optimized systematically. Additionally, the recovery of extracted five target flavonoids directly from tailored-DES extraction solution by macroporous resin was also evaluated. The antioxidant activities of enriched flavonoids from SBL were evaluated by DPPH, ABTS radical-scavenging activity and reducing power assays.

2. Materials and methods

2.1 Materials

SBL were collected from Heilongjiang academy of agricultural science institute of berries in Heilongjiang province, PR China. Voucher specimens were deposited in the herbarium of key laboratory. SBL were cleaned with deionized water and dried in the shade. Thereafter, the dried sample were pulverized, sieved (40 mesh) and stored in a closed desiccator at room temperature prior to use.

Citric acid, malic acid, lactic acid, ethylene glycol, 1,3-butanediol, 1,4-butanediol, 1,6-hexanediol, 1,2-propanediol, glycerol, glucose, fructose, and sucrose were acquired from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Choline chloride (>98.0%) was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Rutin (≥95%, RU), quercetin-3-O-glucoside (≥95%, QOG), quercetin (≥95%, QU), kaempferol (≥95%, KA), isorhamnetin (≥95%, IS), 1,1-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (Vc) and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile and phosphoric acid were used for the mobile phase preparation and purchased from J&K Chemicals Ltd. (Beijing, China). ABTS assay was purchased from Beyotime Institute of Biotechnology (China). Deionized water was prepared by a Milli-Q water system (Bedford, MA, USA).

2.2 HPLC analysis

RU, QOG, QU, KA and IS were simultaneously determined by an Agilent 1200 HPLC system coupled with an Agilent 1200 multiple wavelength detector. Chromatographic separation was performed on a KYA TECH HIQ Sil C18 column (250 mm × 4.6 mm i.d., 5 μm). The chromatographic conditions were conducted as our previous study (Cui et al., 2017). The detection wavelength was 254 nm. The chromatograms of RU, QOG, QU, KA and IS were quantified at 254 nm as shown in Fig. 2. In Table 1, the calibration data, precision and

recovery yields of RU, QOG, QU, KA and IS were determined according to the methods as reported in previous study (Cui et al., 2017).

2.3 Preparation of choline chloride-based DESs

The choline chloride-DESs were prepared by adding different mole ratios of hydrogen bond donors (HBDs) to choline chloride to form a homogeneous transparent liquid at 80 ^{o}C with constant stirring (Table 2). In this study, twelve different kinds of DESs were prepared using inexpensive and natural components as shown in Table 2. The DESs were kept in sealed glass vials in the dark, at ambient temperature until use.

2.4 Extraction procedures

2.4.1 DES-MAE

1.0 g of SBL sample and 20 mL of the prepared DES-6 (3/1, mol/mol) with 20% (v/v) water were put into a microwave reaction flask (50 mL) for microwave-assisted extraction in a MAS-□ Plus (2450 MHz) normal pressure microwave synthesis/extraction response workstation (Sineo Microwave Chemistry Technology Co., LTD, Shanghai, China) which was fixed at 600 W. After 17 min, the mixture was filtered through a 0.45 μm nylon filter and determined by HPLC. Three parallel experiments were performed.

2.4.2 Traditional extraction methods

For DES-HRE and DES-UAE, a reflux device and an ultrasonic bath was applied for extraction of target compounds, respectively. The subsequent step was the same as DES-MAE.

2.5 Experimental design

The extraction variables of DES-MAE were determined systematically by single-factor experiment and Box-Behnken design (BBD) at three levels (1-, 0, +1). Single-factor experiment investigated the DES type, mole ratio of 1,4-butanediol and choline chloride, and water content in DES on the extraction efficiency of target flavonoids in SBL. BBD united with response surface methodology (RSM) was applied to determine the optimal combination of extraction parameters for RU, QOG, QU, KA and IS. Liquid/solid ratio (X_1), extraction temperature (X_2)

and extraction time (X_3) were the key independent variables, the extraction yields of RU (Y_1), QOG (Y_2), QU (Y_3), KA (Y_4) and IS (Y_5) were the response variables. In total, seventeen experimental points including five central points (treatments 13-17) were carried out in random order. The actual and coded levels of the variables used in the experimental design are summarized in Table 3. A second order polynomial model was fitted to the experimental data and to predict the optimal conditions.

2.6 Recovery of SBL target flavonoids

A glass column (15 mm × 500 mm) wet-packed with 10 g (dry weight) of the selected macroporous resin including NKA-9, AB-8 and D101 (Table S1 in the Electronic Supporting Information) was applied for the recovery of bioactive flavonoids in DES extraction solution. 24 mL of the extraction solution obtained under optimal extraction conditions flowed through the column at a constant flow rate of 3 BV/h. After sample loading and adsorption attained equilibrium, 270 mL of deionized water was used to wash the adsorbate-laden column firstly. After that, 270 mL of 95% (v/v) ethanol was applied for eluting the column at a flow rate of 6 BV/h. The 95% (v/v) ethanol elution solution was combined and vaporized with the rotary evaporator. The contents and recoveries of five target flavonoids (RU, QOG, QU, KA and IS) in the effluent liquid were monitored and determined by HPLC as described in above section.

3. Results and discussion

3.1 Selection of DESs

DES, as a unique class of designed multi-component solvent system possessed the advantages of tunability and selectivity for the extraction of natural products from the plant matrix by changing its components. Therefore, a suitable DES type with varying physico-chemical properties including solubility, viscosity, surface tension, polarity and physicochemical interactions is quite important in the process of solid-liquid extraction (Dai et al., 2013; Lu et al., 2016). In this study, twelve different types of choline chloride-based DESs (Table 2) and five ranges of the HBD/ChCl ratio (1/1, 2/1, 3/1, 4/1, 5/1 (mol/mol)) for DES-6 were prepared to extract target flavonoid compounds from SBL in this study. As shown in Fig. 3A, the types of DES had different effects on target compounds extraction yields. It was obvious that the extraction

yields of target flavonoids by the polyalcohols-based DESs and sugar-based DESs were higher than those organic acids-based DESs, which was consistent with the weak and medium polarity of the target compounds (Fig. 3A, Table S2). Among the twelve DESs, the optimal DES which exhibited the highest extraction yields (9.357, 3.792, 8.256, 0.404 and 0.348 mg/g) of five target compounds (RU, QOG, QU, KA and IS) was DES-6 (1,4-butanediol/ChCl). Meanwhile, according to Table S2, 1,4-butanediol/ChCl possessed the minimum viscosity among these DESs as compared with water and methanol, which indicated that DES-6 was preferred to extract the target flavonoids from SBL due to its better penetration of pores in the matrix. In addition, increasing the temperature can decrease the surface tension and viscosity of DES, which led to the increase of extraction yields of target flavonoids. The results obtained in this study was consistent with those in the literature (Bi et al., 2013).

The DESs with different 1,4-butanediol/ChCl ratios were evaluated for the extraction yields of five target flavonoids. As shown in Fig. 3B, with the increased amount of 1,4-butanediol/ChCl ratio from 1/1 to 3/1 (mol/mol), the extraction yields of five target compounds increased significantly. Whereas, the 1,4-butanediol/ChCl ratio attained to 4/1 and 5/1 (mol/mol), the extraction yields of five target compounds decreased apparently (Fig. 3B). According to the previous studies, the viscosity and surface tension of DES would decrease with the increase of HBD ratio in DES as shown in Table S2, which could improve the effect of diffusion and mass transfer (Naser et al., 2013; Cao et al., 2017). However, a sustainable increase of HBD ratio in DES would reduce the interactions between the flavonoids and chloride anion (Qi et al., 2015). Therefore, the DES-6 of 1,4-butanediol/ChCl (3/1, mol/mol) with suitable physio-chemical properties and polarity was selected for the following experiments.

3.2 Effect of water content in DES on extraction efficiency

When compared with conventional solvents, the major disadvantage of DES is their inherent high viscosity which hinders the mass transfer from plant matrix to extraction solution (Cui et al., 2015). Besides, the water content also plays an important role in affecting the viscosity of DES. The presence of water can decrease the viscosity and modulate the polarity, then increase the dissolution rate of flavonoid

compounds (Dai et al., 2013). As shown in Fig. 3C, the effect of water content in DES on the extraction yields of five flavonoid compounds was performed at different water content (0-80%). The results in Fig. 3C suggested that the extraction yields of RU, QOG, QU, KA and IS from SBL improved significantly with increasing proportions of water, attained to a peak value of 8.554, 1.711, 7.957, 0.411 and 0.454 mg/g (20% w/w). Generally speaking, the addition of water could decrease the viscosity of DES, enhance the dissolution efficiency of target compounds, and then significantly increase the extraction efficiency to some extent (Ruesgas-Ramón et al., 2017). However, excessive water content (> 20%) in DES-6 solution was counterproductive to the increase in the extraction yields of five target compounds, which probably was owing to the interactions between DES and target compounds was weakened and broken. We also measured the pH value of DESs with different water content from 0 to 80% (Table S3). It can be found that the changing of pH had little effect on the extraction yields of target flavonoids. The difference in water content of DES resulted in the difference of its viscosity and polarity, which led to the change of extraction efficiency. Therefore, a suitable water content (20%) in 1,4-butanediol/ChCl (3/1, mol/mol) was chosen for extracting flavonoid compounds in subsequent tests.

3.3 Optimization of the operational parameters for flavonoids extraction

The tuneability of DES as a designer solvent to selectively extract target flavonoids from SBL in this study was investigated by single factor experiment. Then three key parameters including liquid/solid ratio, extraction temperature and extraction time affecting the extraction efficiency were investigated and optimized in detail. In the present study, DES-6 and microwave-assisted extraction (MAE) method were chosen as the optimal extraction solvent and extraction method for their key function of extraction efficiency. Pre-experiments were carried out to determine the range of three significant parameters: liquid/solid ratio (15-25 mL/g), extraction temperature (45-75 ^{o}C) and extraction time (10-20 min). So a seventeen-run Box-Behnken (BBD) with three factors and three levels, including five replicates at center point, was employed in order to optimize the extraction conditions, and the mean amounts of corresponding compounds extracted from SBL were taken as the responses. The experimental data were depicted in Table 3 and they were analyzed using Design-Expert 8.0.6 software. A second order

polynomial equation for each flavonoid was obtained by the multiple regression analysis.

$$Y_{\text{rutin}} = 8.65 + 0.49X_1 + 0.74X_2 + 0.54X_3 - 0.29X_1X_2 - 0.26X_1X_3 + 0.12X_2X_3 - 0.90X_1^2 - 1.29X_2^2 - 0.26X_3^2 \quad (1)$$

$$Y_{\text{quercetin-3-O-glucoside}} = 1.76 + 0.10X_1 + 0.13X_2 + 0.13X_3 - 0.027X_1X_2 - 0.003636X_1X_3 - 0.011X_2X_3 - 0.37X_1^2 - 0.25X_2^2 - 0.15X_3^2 \quad (2)$$

$$Y_{\text{quercetin}} = 8.74 + 0.50X_1 + 0.82X_2 + 0.61X_3 - 0.29X_1X_2 - 0.088X_1X_3 + 0.25X_2X_3 - 1.12X_1^2 - 1.48X_2^2 - 0.31X_3^2 \quad (3)$$

$$Y_{\text{kaempferol}} = 0.43 + 0.034X_1 + 0.027X_2 + 0.048X_3 + 0.008505X_1X_2 - 0.003247X_1X_3 + 0.015X_2X_3 - 0.089X_1^2 - 0.12X_2^2 - 0.012X_3^2 \quad (4)$$

$$Y_{\text{isorhamnetin}} = 0.47 + 0.049X_1 + 0.058X_2 + 0.040X_3 + 0.001456X_1X_2 - 0.014X_1X_3 + 0.006464X_2X_3 - 0.11X_1^2 - 0.11X_2^2 - 0.017X_3^2 \quad (5)$$

Where Y was the extraction yield of target flavonoid (mg/g); X_1, X_2 and X_3 represented liquid/solid ratio (mL/g), extraction temperature (^{o}C) and extraction time (min), respectively.

The ANOVA indicated that quadratic regression models for obtaining five target flavonoids were adequate, with satisfactory R^2 value and no significant lack of fit. The significance of each coefficient was checked using F-test and P value (Table 4). High F-value (more than 29.80) and low p-value ($p < 0.0001$) showed that the models for five target flavonoids were suitable for the extraction process. The regression analysis of the data showed the coefficient of determination (R^2) values were 0.9746 or higher in the five models, indicating the experimental data were in excellent agreement with predicted models. The "Lack of Fit F-value" of less than 6.6 indicated the Lack of Fit was not significant, which indicated that the models built could accurately represent the experimental data. Moreover, coefficient of variation (C.V.) with a low value of less than 5.96 showed a high degree of precision and a good deal of reliability of the experimental data. Meanwhile, as shown in Table 4, the linear coefficients of X_1, X_2 and X_3 and quadratic term coefficients of X_1^2, X_2^2 and X_3^2 were all significant. Therefore, the liquid/solid ratio (X_1), extraction temperature (X_2) and extraction time (X_3) were important factors in the extraction process of the target flavonoids.

The three-dimensional (3D) response surface plots were used to visualize the relationship between the responses and experimental levels

of each variable and to illustrate the interaction effects between the variables affecting extraction yields of the flavonoids. In this study, the extraction yields of target flavonoids (RU, QOG, QU, KA and IS) were highly affected by liquid/solid ratio (X_1), extraction temperature (X_2) and extraction time (X_3), as shown in Fig. 4. Fig. 4A and E showed interaction on the extraction yields of RU and IS of liquid/solid ratio (X_1) and extraction temperature (X_2), respectively. As could be observed that the extraction yields increased rapidly with an increase in the liquid/solid ratio from 15 to 21 mL/g and extraction temperature from 45 to 64 ^{o}C. Generally, the extraction yields for target flavonoids increased with higher liquid/solid ratio and higher extraction temperature. The extraction yields increased with increasing extraction temperature until the extraction temperature reached 64 ^{o}C, but an excessively high temperature decreased the yields of flavonoids. As illustrated in Fig. 4B and C, the yields of QOG and QU increased sharply to a maximal value with the increase in liquid/solid ratio from 15 to 21 mL/g along with an increase in extraction time from 10 to 17 min, while began to decrease when the liquid/solid ratio and extraction time surpassed 21 mL/g and 17 min. According to previous research, higher solvent volume used would augment the energy consumption of microwave and affect the extraction yields of target compounds (Duan et al., 2013). Fig. 4D showed that the extension of extraction time had a promoting effect on enhancing the extraction yield of KA. When the extraction time was above 17 min, the extraction yield had no significantly change. From the above results, it can be confirmed that appropriate extraction conditions were more conductive to effectively extract target flavonoids (RU, QOG, QU, KA and IS) and natural products from SBL and plant biomass.

The theoretical maximal extraction yields of RU, QOG, QU, KA and IS (8.976, 1.814, 9.112, 0.456 and 0.496 mg/g) were predicted under the following conditions: liquid/solid ratio 20.62 mL/g, extraction temperature 63.81 ^{o}C and extraction time 17.09 min, respectively. Taking convenience into account, liquid/solid ratio 21 mL/g, extraction temperature 64 ^{o}C and extraction time 17 min were selected as the optimum experimental parameters for extraction of five target flavonoids from SBL by 1,4-butanediol/ChCl (3/1, mol/mol) with MAE. The actual extraction yields of RU, QOG, QU, KA and IS were 8.967, 1.809, 9.107, 0.448 and 0.489 mg/g with calculated RSD less than 1.77% under the optimum experimental parameters, respectively, which were consistent with the theoretical values. The results of the verification experiments

indicated that the established models were reliable and reasonable.

3.4 Comparison of different extraction methods

Different extraction methods and solvents used for the extraction of flavonoids from SBL were performed and compared (Table 5). It was observed that the extraction yields of RU, QOG, QU, KA and IS by NADES-MAE were 1.082-1.587, 1.192-1.625 and 1.697-3.069 folds to those by DES-UAE, DES-HRE and ethanol-MAE. The excellent extraction efficiency of MAE was attributed to the microwave irradiation which can significantly accelerate the extraction process, shorten the extraction time and increase extraction yield. Meanwhile, the interaction between DES and target flavonoids made DES possess preferable extraction efficiency. Consequently, the current extraction method DES-MAE was definitely an efficient and eco-friendly extraction method with enhanced efficiency, which could be applied for the extraction of natural bioactive compounds from plant matrix.

3.5 Recovery of SBL target flavonoids from DES

The process of conventional enrichment of natural products from extraction solution with macroporous resin generally requires the concentration and retreatment of extraction solution, which not only call for energy- and time-consuming, the target compounds is prone to decompose as well (Wei et al., 2015; Cao et al., 2017). In the present work, three macroporous resin NKA-9, AB-8, and D101 were evaluated to recover five target flavonoids from DES extraction solution. The adsorption and desorption performances of resins were shown in Fig. S1 (Electronic Supporting Information). It could be found that the adsorption capacities and desorption ratios were not only correlated with physicochemical properties of the resins, but also were influenced by the size and chemical features of the adsorbed substance. From the results shown in Table 6, it was observed that the weak-polar macroporous resin AB-8 exhibited better performance than the others. After one run treatment with AB-8 resin, the contents of RU, QOG, QU, KA and IS enriched by AB-8 resin reached 9.74%, 4.67%, 9.35%, 3.07% and 4.72%, and the recovery yields were 83.59%, 74.46%, 84.99%, 72.36% and 77.85%, respectively. It was indicated that AB-8 resin possessed excellent capacity for enriching five target flavonoids from DES extraction solution. The five target flavonoids could be absorbed and recovered by AB-8

resin, while the DES could be eluted with deionized water. In hence, AB-8 macroporous resin was suitable and efficient for the enrichment and recovery of five target flavonoids from DES. The recycling of DES was also an important issue to be investigated. In the recovery of SBL target flavonoids in DES extraction solution, the loaded DES extraction solution was washed with deionized water. The effluent solution and washing water were combined together, excess water was removed to get DES liquid phase by evaporating under vacuum (Jeong et al., 2015). The regenerated solvent was then used for extraction under the optimized conditions. The recycling of DES could be used for the extraction at least four times with the yields of 18.769, 17.966, 17.029 and 14.070 mg/g, respectively (Fig. S2 in Electronic Supporting Information). The recovery yields of the recycled DES could be obtained from 76.38% to 91.15%. These results indicated that the DES could be recycled to achieve a reasonably high level of target flavonoids yields from sea buckthorn leaves.

3.6 Antioxidant activities of enriched flavonoids from sea buckthorn leaves

As a kind of effective antioxidants, flavonoids have attracted a widespread attention (Wu et al., 2009; Wu et al., 2010). Therefore, the antioxidant activities of the enriched flavonoids by macroporous resins (NKA-9, AB-8 and D101) were measured and compared by DPPH, ABTS radical-scavenging activity and reducing power assays. As shown in Table 7, the enriched flavonoids by AB-8 resin exhibited better DPPH antioxidant activity with IC_{50} value of 0.074 mg/mL, which was superior to those by NKA-9 (0.091 mg/mL) and D101 (0.131 mg/mL). ABTS radical-scavenging activity assay is applied for measuring the total antioxidant capacity of samples. It was indicated in Table 7, the TEAC values from highest to lowest was AB-8 (0.662 mmol/g Trolox) > D101 (0.497 mmol/g Trolox) > NKA-9 (0.461 mmol/g Trolox), which was lower than the value of Vc (1.034 mmol/g Trolox). In the reducing power assay, the enriched flavonoids by AB-8, D101 and NKA-9 revealed good reducing power with the IC_{50} values of 0.127, 0.184 and 0.209 mg/mL, respectively. The IC_{50} value of butylated hydroxytoluene (BHT) was 0.136 mg/mL. Overall, the antioxidant activity of enriched flavonoids by AB-8 resin was significantly higher than those by the other two resins and the extracts reported previously (Cui et al., 2017), which demonstrated that SBL could be a valuable natural antioxidant source and the potential to be applicable in both nutritional and medicinal industry.

3.7 DES-MAE pilot-scale application

The objective of this work was to evaluate the potential applications of DES-MAE in the industrial production. Hence, an experimental DES-MAE pilot-scale application with 500 g SBL materials were subjected to extraction under the optimal conditions (liquid/solid ratio 21 mL/g, extraction temperature 64 ^{o}C and extraction time 17 min) for three times and compared with the predicted values obtained in Section 3.3. The observed extraction yields of RU, QOG, QU, KA and IS using pilot-scale were 8.972, 1.821, 9.104, 0.445 and 0.493 mg/g, respectively, which were in accordance with the predicted ones. The results showed that the suitability and reliability of the established quadratic models for the extraction process. Regarding on the costs and environmental effect, the proposed method DES-MAE demonstrated many green and sustainable impacts which could shorten extraction time, improve extraction efficiency, consume less energy. Comprehensive consideration of these aspects mentioned above, the developed DES-MAE method was a potential and sustainable alternative technique to traditional extraction methods in extraction of natural compounds from plant matrices that can be scaled up for industrial application.

4. Conclusions

In the present study, an efficient and green extraction method named DES-MAE was first described for flavonoids extractions from sea buckthorn leaves. The DES-6, 1,4-butanediol/ChCl (3/1, mol/mol) was tailor-made solvent system to provide the highest extraction efficiency of rutin, quercetin-3-O-glucoside, quercetin, kaempferol and isorhamnetin, after a systematic screening and comparison of ChCl-based DESs type and HBD/ChCl ratio. Under the optimized conditions: liquid/solid ratio 21 mL/g, extraction temperature 64 ^{o}C and extraction time 17 min, the scale-up extraction yields of RU, QOG, QU, KA and IS using pilot-scale were 8.972, 1.821, 9.104, 0.445 and 0.493 mg/g, respectively. Low cost and high efficiency are of great value for the extraction of natural products from plant matrix. Compared with DES-HRE and DES-UAE, DES-MAE exhibited the higher extraction yields of target flavonoids in shorter extraction time, which consumed less energy and less impact on the environment. DES as a designer solvent for the extraction of target flavonoids from SBL in this study, possessed incomparable performance, which made it predominant to traditional toxic organic reagents. Moreover, the enrichment and recovery of five target flavonoids from DES

extraction solution was readily and directly performed by macroporous resin AB-8 with excellent recovery more than 72.36%. The recycling of DES could be used for the extraction at least four times with the yields and recovery yields of more than 14.070 mg/g and 76.37%. In addition, the enriched flavonoids by macroporous resin possessed higher antioxidant activity than crude extracts. Therefore, DES-MAE coupled with macroporous resin enrichment procedure, could be an excellent sustainable and efficient alternative for obtaining natural products from plant matrices in pharmaceutical, biochemical, and food industries.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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Figure Captions

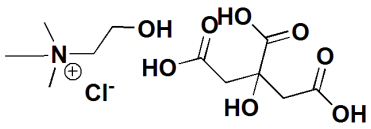
Figure 1 Structure of DESs based on choline chloride.

Figure 2 HPLC chromatograms of sea buckthorn leaves crude extracts (blue) and enriched by macroporous resin (black) at 254 nm: (1) RU, (2) QOG, (3) QU, (4) KA, (5) IS.

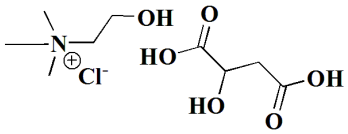
Figure 3 Effect of the types of DESs (A), 1,4-butanediol/ChCl ratio (B) and water content in DESs (C) on the extraction yields of RU, QOG, QU, KA and IS from sea buckthorn leaves.

Figure 4 Response surfaces representations for RU (A), QOG (B), QU (C), KA (D) and IS (E) in sea buckthorn leaves. A and E varying liquid/solid ratio and extraction temperature; B and C varying liquid/solid ratio and extraction time; D varying extraction temperature and extraction time.

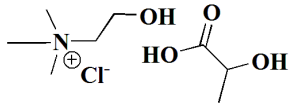
Fig. 1 Structure of DESs based on choline chloride.



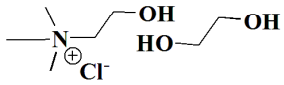
Citric acid/ChCl (1:1)



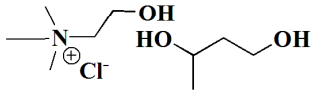
Malic acid/ChCl (1:1)



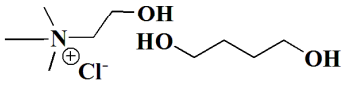
Lactic acid/ChCl (1:1)



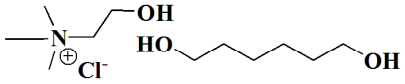
Ethylene glycol/ChCl (1:1)



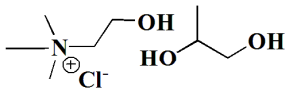
1,3-butanediol/ChCl (1:1)



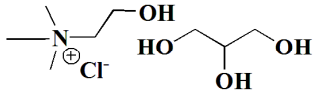
1,4-butanediol/ChCl (1:1-5:1)



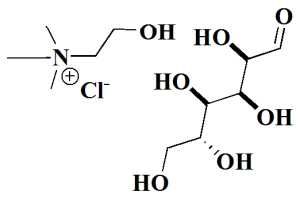
1,6-hexanediol/ChCl (1:1)



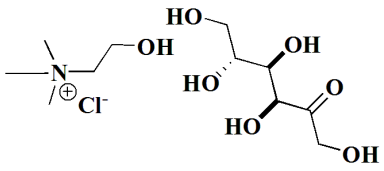
1,2-propanediol/ChCl (1:1)



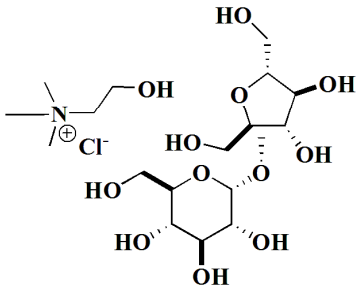
Glycerol/ChCl (1:1)



Glucose/ChCl (1:1)

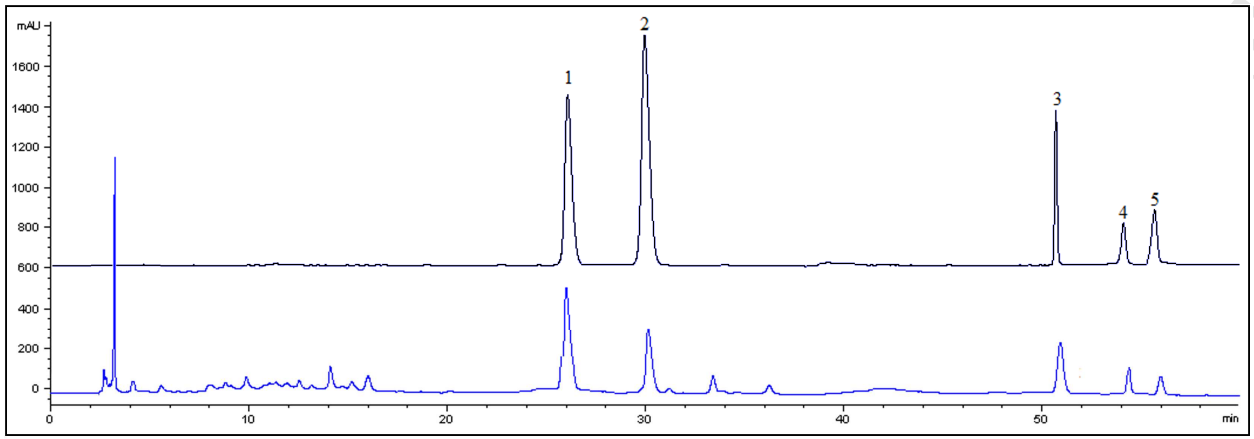


Fructose/ChCl (1:1)



Sucrose/ChCl (1:1)

Fig. 2 HPLC chromatograms of sea buckthorn leaves crude extracts (blue) and enriched by macroporous resin (black) at 254 nm: (1) RU, (2) QOG, (3) QU, (4) KA, (5) IS.



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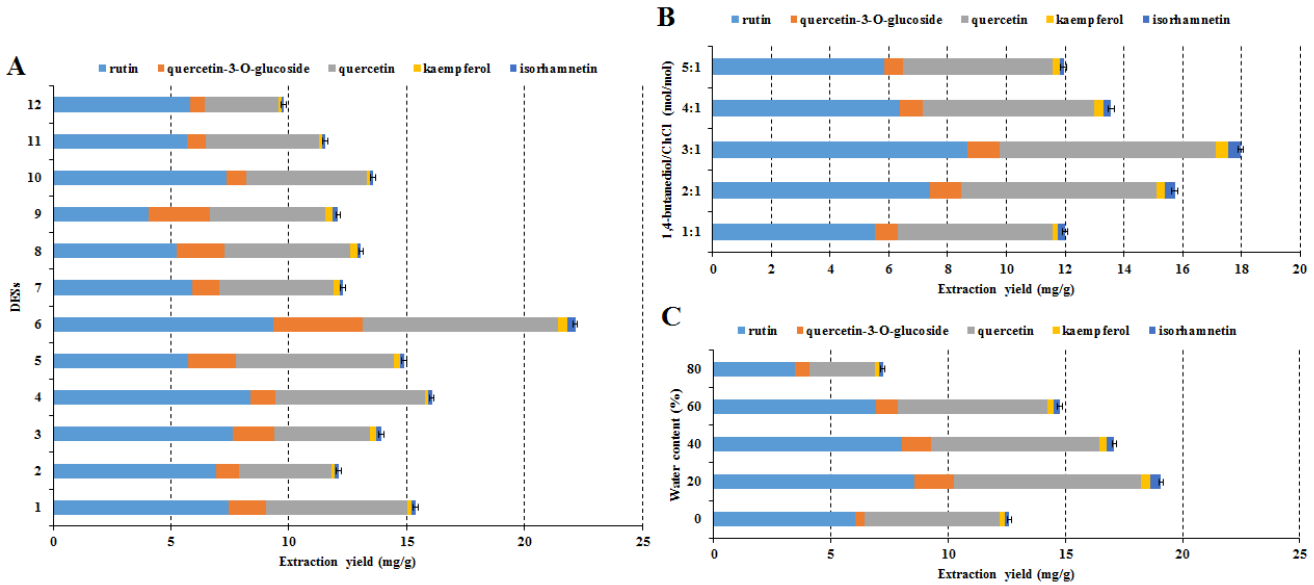

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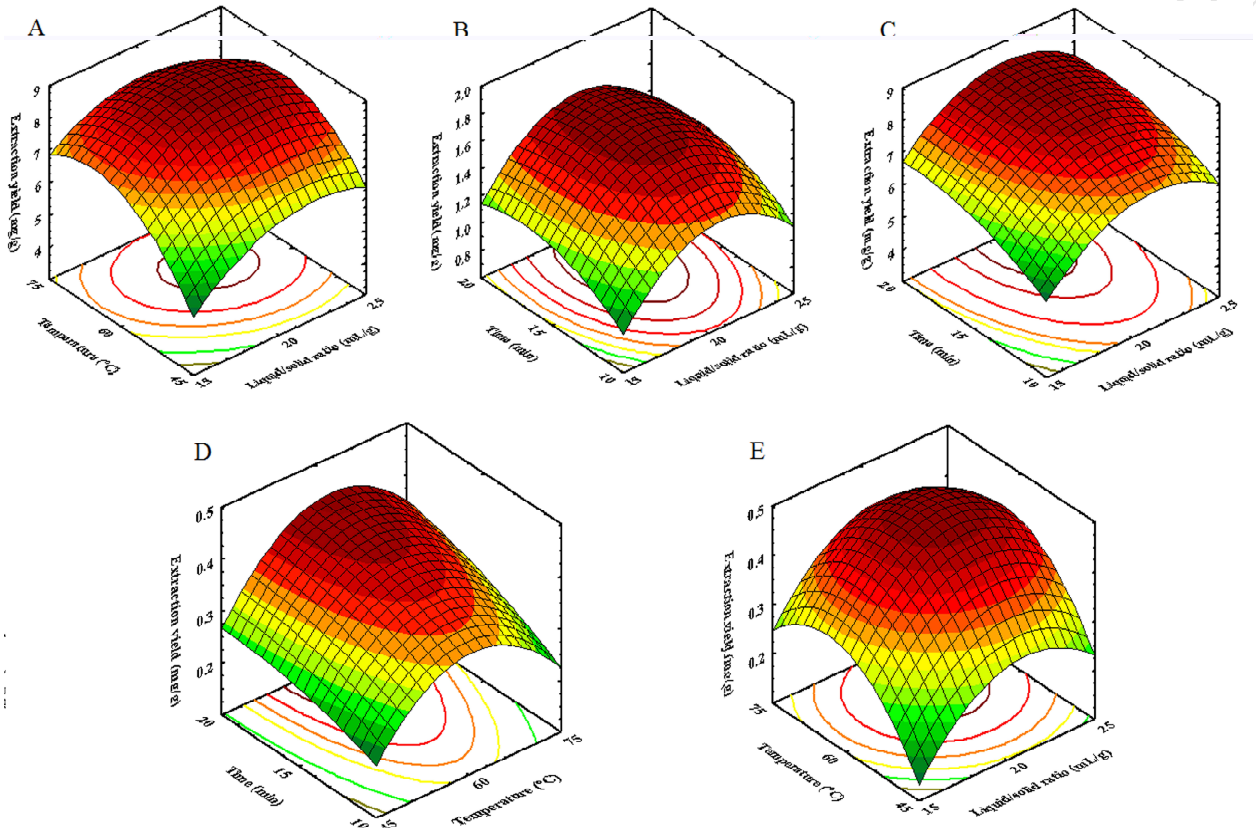

Table 1 Calibration data, precision and recovery yields of five bioactive compounds (n = 6).

Analyte	Linearity range (μg/mL)	LOD (μg/mL)[a]	LOQ (μg/mL)[a]	Intra-day RSD		Inter-day RSD		RSD		Amount added (mg)	Recovery (%)	RSD (%)
				Pa (%)	Rt (%)	Pa (%)	Rt (%)	Pa (%)	Rt (%)			
RU	2.5-500	0.38	1.45	3.42	0.57	3.61	0.51	3.62	0.52	0.196	98.72	2.95
										0.392	98.33	3.12
QOG	2.5-500	0.46	1.74	2.83	0.43	3.02	0.47	2.84	0.69	0.302	99.12	3.68
										0.604	98.67	3.27
QU	2.5-500	0.28	0.89	2.67	0.35	2.93	0.39	3.15	0.78	0.255	98.26	2.89
										0.510	97.87	3.23
KA	2.5-500	0.35	1.15	3.29	0.41	3.16	0.45	2.49	0.43	0.276	99.26	3.58
										0.552	98.85	3.24
IS	2.5-500	0.41	1.27	2.74	0.39	2.53	0.54	2.93	0.49	0.329	98.40	2.97
										0.658	99.13	3.54

[a] LOD refers to the limit of detection, LOQ refers to the limit of quantification.

Table 2 Different systems of the prepared DESs.

Abbreviation	HBD	Salt	Mole ratio	Appearance	Reference
DES-1	Citric acid	Choline chloride	1:1	Transparent liquid	Abbott et al. (2004)
DES-2	Malic acid		1:1	Transparent liquid	Dai et al. (2013)
DES-3	Lactic acid		1:1	Transparent liquid	Dai et al. (2013)
DES-4	Ethylene glycol		1:1	Transparent liquid	Abbott et al. (2007)
DES-5	1,3-butanediol		1:1	Transparent liquid	Bi et al. (2013)
DES-6	1,4-butanediol		1:1, 2:1, 3:1, 4:1, 5:1	Transparent liquid	Abbott et al. (2007)
DES-7	1,6-hexanediol		1:1	Transparent liquid	Bi et al. (2013)
DES-8	1,2-propanediol		1:1	Transparent liquid	Dai et al. (2013)
DES-9	Glycerol		1:1	Transparent liquid	Abbott et al. (2007)
DES-10	Glucose		1:1	Faintly yellow liquid	Dai et al. (2013)
DES-11	Fructose		1:1	Faintly yellow viscous liquid	Ilgen et al. (2009)
DES-12	Sucrose		1:1	Faintly yellow viscous liquid	Dai et al. (2013)

Table 3 Results of the Box-Behnken design (BBD) for the extraction yields of RU, QOG, QU, KA and IS.

Runs	Factors			Extraction yield (mg/g DW)				
	X_1 (L[a], mL/g)	X_2 (Tem[b], ^oC)	X_3 (t[c], min)	Y_1	Y_2	Y_3	Y_4	Y_5
1	-1(15)	-1(45)	0(15)	4.872	0.831	4.452	0.172	0.142
2	1(25)	-1(45)	0(15)	6.573	1.114	6.184	0.227	0.252
3	-1(15)	1(75)	0(15)	6.915	1.232	6.680	0.216	0.242
4	1(25)	1(75)	0(15)	7.475	1.406	7.270	0.305	0.358
5	-1(15)	0(60)	-1(10)	6.230	0.984	6.165	0.254	0.237
6	1(25)	0(60)	-1(10)	7.584	1.172	7.182	0.323	0.347
7	-1(15)	0(60)	1(20)	7.920	1.324	7.628	0.349	0.374
8	1(25)	0(60)	1(20)	8.219	1.497	8.292	0.341	0.428
9	0(20)	-1(45)	-1(10)	5.971	1.181	5.841	0.246	0.255
10	0(20)	1(75)	-1(10)	7.218	1.386	6.943	0.264	0.372
11	0(20)	-1(45)	1(20)	6.732	1.377	6.475	0.320	0.292
12	0(20)	1(75)	1(20)	8.471	1.540	8.582	0.398	0.435
13	0(20)	0(60)	0(15)	8.647	1.737	8.782	0.432	0.468
14	0(20)	0(60)	0(15)	8.640	1.745	8.818	0.424	0.459
15	0(20)	0(60)	0(15)	8.626	1.848	8.696	0.425	0.491
16	0(20)	0(60)	0(15)	8.595	1.737	8.684	0.449	0.463
17	0(20)	0(60)	0(15)	8.757	1.751	8.731	0.440	0.470

[a] The liquid/solid ration (mL/g).

[b] The extraction temperature (^oC).

[c] The extraction time (min).

Table 4 ANOVA statistics of the quadratic model for the extraction yields of RU, QOG, QU, KA and IS.

Source	RU		QOG		QU		KA		IS	
	F-value	p-value	*F*-value	p-value	*F*-value	p-value	*F*-value	p-value	*F*-value	p-value
Model	221.62	< 0.0001	29.80	< 0.0001	272.81	< 0.0001	147.92	< 0.0001	42.42	< 0.0001
X_1	182.19	< 0.0001	16.29	0.0050	182.46	< 0.0001	92.24	< 0.0001	3.10	0.0003
X_2	418.33	< 0.0001	27.44	0.0012	484.60	< 0.0001	59.93	0.0001	4.08	0.0001
X_3	223.99	< 0.0001	25.04	0.0016	267.30	< 0.0001	188.46	< 0.0001	5.11	0.0011
X_1^2	326.02	< 0.0001	113.76	< 0.0001	480.74	< 0.0001	338.73	< 0.0001	20.12	< 0.0001
X_2^2	669.04	< 0.0001	49.54	0.0002	835.93	< 0.0001	567.19	< 0.0001	47.79	< 0.0001
X_3^2	27.70	0.0012	17.72	0.0040	35.90	0.0098	6.14	0.0423	23.19	0.1409
X_1X_2	30.97	0.0008	0.58	0.4720	29.73	0.0010	2.94	0.1300	0.82	0.8950
X_1X_3	26.49	0.0013	0.01	0.9220	2.84	0.1358	0.43	0.5335	0.69	0.2187
X_2X_3	5.76	0.0475	0.089	0.7746	22.99	0.0020	9.12	0.0194	2.12	0.5628
Lack of fit	5.18	0.0729	3.95	0.1090	6.57	0.0503	0.68	0.6095	5.66	0.0637
R^2	0.9965		0.9746		0.9972		0.9948		0.9820	

Table 5 Comparison of different extraction method on the extraction yields of RU, QOG, QU, KA and IS from SBL.

Extraction method	Extraction yield (mg/g DW)					Extraction condition		
	RU	QOG	QU	KA	IS	Temperature (^{o}C)	Power (W)	Time (min)
DES-MAE	8.967	1.809	9.107	0.448	0.489	64	600	17
DES-UAE	7.158	1.672	5.739	0.303	0.396	64	250	45
DES-HRE	6.734	1.517	6.845	0.368	0.301	64	-	45
Ethanol-MAE	4.374	0.871	2.967	0.264	0.198	64	600	17

Table 6 The enrichment results of five target compounds with three macroporous resins.

Trade name	Mass of extracts (mg/g DW)[a]	Composition	Contents (%)	Recovery yields (%)
NKA-9	109.35	RU	7.25	87.76
		QOG	4.11	72.51
		QU	7.98	83.57
		KA	2.17	65.43
		IS	3.46	60.12
AB-8	134.48	RU	9.74	83.59
		QOG	4.67	74.46
		QU	9.35	84.99
		KA	3.07	72.36
		IS	4.72	77.85
D101	118.57	RU	5.16	80.12
		QOG	2.94	64.38
		QU	6.03	70.47
		KA	2.24	65.23
		IS	3.58	68.26

Table 7 Comparison of antioxidant activity with different extracts after enrichment.

Trade name	DPPH IC_{50} (mg/mL)	ABTS (mmol/g Trolox)	Reducing power IC_{50} (mg/mL)
NKA-9	0.091	0.461	0.209
AB-8	0.074	0.662	0.127
D101	0.131	0.497	0.184
V_C	0.064	1.034	-
BHT	-	-	0.136

Highlights:

- A range of sustainable and green DES were prepared and synthesized successfully.
- DES-MAE process exhibited excellent skills in terms of flavonoids extraction.
- The target flavonoids in DES extraction solution can be efficiently recovered.
- The antioxidant activity of SBL extract was significantly enhanced by AB-8 resin.