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Deep eutectic solvent strategy for green extraction of chlorogenic acid from sea buckthorn: optimization and sustainability

Iqra Saddique¹, Sumia Akram², Saima Rubab³, Ayesha Sadiqa⁴, Ali Raza⁵, Muhammad Mushtaq^{1*} and Mohsin Ahmad Ghauri^{3*}

Abstract

Background Sea buckthorn (*Hippophae rhamnoides*), a deciduous species plant, is widely distributed around the globe, and native to the cold-temperate regions of Europe and Asia. This medicinal herb contains several bioactive constituents including chlorogenic acid. The conventional methods used for the extraction of phenolic antioxidants from natural herbs often result in low yields, high toxicity, and pose environmental hazards limiting their effectiveness and scalability. Therefore, green extraction techniques using deep eutectic solvents, composed of natural, non-toxic, and biodegradable components were applied for extraction of chlorogenic acid from sea buckthorn weed. Fourteen deep eutectic solvent mixtures were prepared and evaluated for extraction yield of chlorogenic acid. Parameters such as hydrogen bond donor-to-hydrogen bond acceptor ratio, liquid-to-solid ratio, shaking speed, and shaking time were optimized for the best mixture.

Results The combination of lactic acid and maltose (1:1) was found to give best extraction yield using response surface methodology. The deep eutectic solvent system under optimum conditions produced 12.2 g/100 g of crude extract sea buckthorn containing 174.7 mg gallic acid equivalents (mg GA)/g of extract. Moreover, the optimized extract exhibited appreciable radical scavenging capacity (91%), trolox equivalent antioxidant capacity (11.2% of extract), and inhibition of peroxide in linoleic acid (80.6%). High-performance liquid chromatography-based characterization revealed the extracts contained chlorogenic acid (20.1 mg/g of extract) as the major constituent.

Conclusions In summary, the adoption of DES for the extraction of bioactive phenolic constituents from sea buckthorn offers multiple benefits, including economic efficiency, enhanced extraction performance, and environmental sustainability. The findings of this study not only advance the understanding of DES in phytochemical extraction but also pave the way for broader application of green solvents in the natural products industry. Future research should focus on further optimizing DES formulations and scaling up the extraction process to fully realize the potential of this innovative extraction method in commercial applications.

Keywords Chlorogenic acid, Sea buckthorn, Green extraction, Antioxidant

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Background

Plants have always held a pivotal role in medicinal practices [1]. Phytotherapy encompasses a broad spectrum of treatment protocols addressing numerous pathological conditions. Beyond providing nutritional value, plant flora presents viable treatment alternatives to available synthetic agents. Plants primarily serve as reservoirs for naturally occurring bioactive compounds, notably primary and secondary metabolites [2, 3]. Chlorogenic acid (CGA) (Fig. 1 Structural formula of CGA) stands out as one of the potent secondary metabolites for its remarkable antioxidant properties [4]. The nomenclature ‘chlorogenic acid’ finds its origin in a Greek term denoting ‘light green’. The CGA constitutes a class of phenolic compounds resulting from the esterification of caffeic acid with quinic acid [5, 6]. This compound confers various pharmacological advantages, encompassing, but not limited to anti-inflammatory, antioxidant, anti-bacterial, and anti-obesity properties [7, 8]. Notably, coffee stands out as a major source of CGA [9]. Additionally, CGA is abundantly present in various food sources such as apples [10], tomatoes, papaya [11], sweet potatoes [12], cabbage, cherry [13], apricots [14], and oranges [15], among others. Marketed under the name “svetol,” CGA is promoted as a dietary supplement, particularly valued for its diverse health benefits, especially in combating obesity [16]. Scientific investigations have validated its antioxidant [17], anti-inflammatory [18], cardiovascular [19], hepatoprotective [20], reno-protective [21], anti-diabetic, and anti-lipidemic potential [22].

Prominent sources of CGA include *Pimpinella anisum*, *Baccharis genistelloides*, *Ilex paraguariensis*, *Achyrocline satureioides*, *Cymbopogon citratus*, *Camellia sinensis*, *Melissa officinalis*, and *Hippophae rhamnoides* [23]. Sea buckthorn berry, a member of the Elaeagnaceae family, thrives in the cold-temperate regions of Europe and Asia. In Asia, *Hippophae rhamnoides* is exclusively distributed in the northern regions of China and throughout the Himalayan region, surrounding India, Nepal, Bhutan,

Pakistan, and Afghanistan [24]. Within the family, which includes 7 species and 11 subspecies, *H. rhamnoides* is commonly known as sea buckthorn, sea berry, or sand thorn. Notably, sea buckthorn fruit exhibits a distinctive yellow to orange–red coloration and a round or oval shape.

Sea buckthorn harbors a spectrum of bioactive components, including tocopherols such as α -tocopherol (121–223 mg/100 g of oil), β -tocopherol (8–12 mg/100 g of oil), γ -tocopherol (127–177 mg/100 g of oil), carotenoids (6–23 mg/100 g of fresh fruit weight), lycopene (8 mg/100 g of fruit), and vitamin C. Furthermore, it boasts asparagine content at 427 mg/100 g of fruit, along with proline at 45 mg/100 g, threonine at 28 mg/100 g, and serine at 37 mg/100 g. The flavonoid content ranges from 212 to 407 mg/100 g of fresh berry weight. Additionally, phenolic chemicals are present in concentrations spanning from 260 to 490 mg per 100 g of fresh berry weight [25, 26]. Historically recognized for its medicinal utility, sea buckthorn has been acknowledged under various monikers such as the “wonderful plant,” “sugar food,” “magic plant,” “bank of vitamins,” and “fundamental food.” In the realm of medicine, the berry serves multifaceted roles as an antioxidant, cytotoxic agent, and a remedy for cardiovascular complications. Furthermore, it has been documented to enhance the immune system, address gastrointestinal ulcers, and mitigate various liver ailments [27, 28]. The sea buckthorn berry in different duration of time is shown in Supplementary Fig. 1 [29].

In recent years, researchers in the field of green chemistry have focused their efforts toward developing and utilizing eco-friendly solvents [30]. Ionic solvents, owing to their distinctive physical and chemical attributes such as low vapor pressure and high boiling point, have garnered considerable attention as environmentally friendly alternatives [31]. However, limitations documented in the literature include concerns related to toxicity, high cost, and poor biodegradability [32]. To address the constraints and limitations associated with ionic liquids (ILs), a novel class of solvents, known as deep eutectic solvents (DES), have emerged [33]. A eutectic mixture represents the specific composition of at least two solid components that undergo a phase transition to a liquid state at a defined temperature. The DES represent a category of ionic fluids typically composed of two or three substances capable of self-association, resulting in the formation of a eutectic mixture characterized by a melting point lower than each individual constituent as shown in Supplementary Fig. 2. The DES serving as eco-friendly solvents are renowned for their ease of preparation, cost-effectiveness, and more importantly biodegradability [34]. DES are often derived from natural and renewable components, making them environmentally

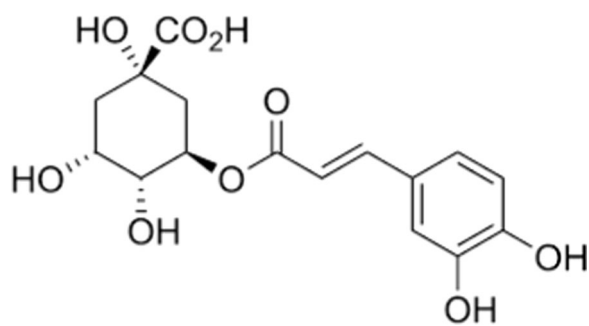


Fig. 1 Chemical structure of chlorogenic acid ($C_{16}H_{18}O_9$)

friendly alternatives to traditional solvents. They contribute to the development of sustainable and green processes. They exhibit low toxicity, are biodegradable, and possess excellent solubility for wide range of compounds. They also carry tunable properties, and in some cases, DES can serve as more sustainable alternatives to ionic liquids, offering similar advantages in terms of green and efficient chemistry.

Operating at ambient temperature, DES involve an acid functioning as the hydrogen bond donor (HBD) and a salt serving as the hydrogen bond acceptor (HBA). Notably, the melting point of DES is lower than that of either of its constituent parts. Natural DES as an effective media for extracting bioactive compounds, aiming to mitigate hazardous waste, enhance selectivity, and improve extraction efficiency have been reported [35, 36]. The reported number of DES derived from plants exceeds 100, demonstrating their remarkable solvation capacity surpassing that of water [37, 38].

The primary objective of present study was to delineate the preparation of diverse DES tailored for the extraction of CGA from sea buckthorn berry without compromising their antioxidant potential. The optimization of key parameters was done using response surface methodology, and the resulting extract's antioxidant activity is systematically evaluated through standard assessment methods. A schematic about experimental design is demonstrated in following diagram (Fig. 2).

Methods

Chemicals and reagents

Deionized water, (Milli-Q-Plus systems), acetamide (HPLC Purity 99% Riedel-de Haën, Switzerland), acetic

acid (United Scientific Supplies, Inc., USA), citric acid (New Chemicals Private Ltd.), D-galactose (Perking Chemicals), D-fructose (New Chemicals Private Ltd), D-glucose dextrose; (Prince Scientific, India), glycine amino acetic acid; (Merck, Germany), L(+)-lactic acid (HPLC Purity: 88–92% Panreac Quimica S.A, Spain), tartaric acid L-(+)-tartaric acid (new chemicals private Ltd), sucrose (Fisher Scientific USA), urea (HPLC Purity 98% Sigma-Aldrich, USA), thiourea purity 98.5% (Sigma-Aldrich), hydrochloric acid (HCl) (United Chemicals, England), potassium hydroxide (Merck, Germany), maltose (Sigma-Aldrich), ethanol (High Purity Chemicals, Japan), sodium carbonate (High Purity Chemicals, Japan), sodium hydroxide (High Purity Chemicals, Japan), trichloroacetic acid (High Purity Chemicals, Japan), ammonium thiocyanate (High Purity Chemicals, Japan), potassium ferrocyanide (High Purity Chemicals, Japan), ferric chloride (High Purity Chemicals, Japan), linoleic acid 99% of purity (Sigma-Aldrich), gallic acid purity 97% (Sigma-Aldrich), Folin–Ciocalteu reagent (Sigma-Aldrich), L-ascorbic acid purity 99% (Sigma-Aldrich), butylated hydroxytoluene purity 99% (Sigma-Aldrich USA).

Equipment

Precision weight balance (Sartorius Gottingen, Model 423-IS, Min 0.001 g, Max 420 g), Ika Vortex, 3 Shaker V3 S1 (747), Water Bath (Mettler, USA), Reflux Condenser, Hot Plate/Stirrer (UTECH Products Inc. Albany, NY 12203, USA), Spectrophotometer (Shimadzu, Japan, equipped with CPS control), Deep Freezer (Waves WCC Model-2150, single door), Autoclave (Omron, Japan), Microplate Stirrer (Clifton), Centrifuge (Hema 1412 Medical instrument, High speed centrifuge), HPLC system (Shimadzu LC 20A, Pump LC20AT, Detector SPD-M20A Diode Array, Injector, Rheodyne, Software LC Solution C-18), Nylon Filters (Micro pore size 0.45 µm) Made by Sartorius (Germany), Sonicator (Elma E-30 Elma sonicator), Vacuum pump (Ulvac Sinku Kiko, Model DA-60D), glass apparatus (Pyrex).

Sample collection and treatment

The berry of plant sea buckthorn was purchased from a local market in Gilgit-Baltistan. The specimen of the plant part was identified as *Hippophae rhamnoides* by taxonomist at GCU Lahore and a sample was also submitted there in the herbarium with voucher number 6875F/Bot. The dried seeds of sea buckthorn were ground to form homogenous powder according to previously described methods with slight modification [36]

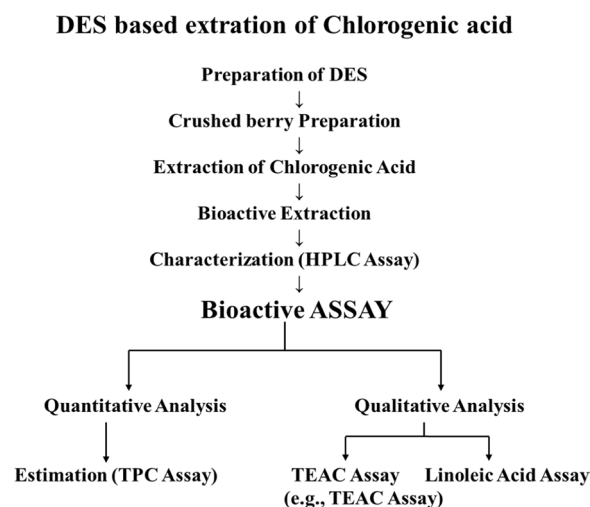


Fig. 2 Schematic representation of experimental design for DES-based extraction of chlorogenic acid from sea buckthorn

DES-based extraction

The first trail was run using water as extracting solvent followed by the preparation of fourteen DES mixtures for extraction (Table 1). In a total of fifteen Erlenmeyer flasks, approximately 2 g of grounded sea buckthorn seed powder along with 30 mL (1:1) of various combinations of DES mixtures was added in respective marked flasks.

The flasks were agitated for 2 h at 175 rpm on a vortex mixer. The extracted contents were filtered by using Whatman filter paper no.1 as shown in Fig. 3.

The filtrate was subjected to drying by means of water bath at temperature of 60 °C. The combinations-containing glucose appears darker, whereas glycine-containing samples appear more reddish. Finally, the percentage yield of each trail was calculated by using Eq. (1).

$$\text{Yield}(\%) = \frac{x_1 - x_2}{m} \times 100$$

(1)

where x_1 and x_2 denote the weights of the beaker containing dried extracts and empty beaker (till no further weight loss), respectively, while m indicates total weight of initial powder taken for extraction.

Optimization of experimental design by response surface methodology (RSM)

After selecting the best combination of DES, the extraction of CGA from sea buckthorn berry was carried out under conditions of different reaction parameters, e.g., shaking speed, H–D/H–Accep ratio, liquid-to-solid ratio, and total extraction time. To achieve an optimum extraction yield of CGA, these reaction parameter optimizations are considered highly significant. The RSM was utilized for simulation of variations of percentage extraction for 21 samples run along with Box–Behnken design (BBD). To predict and evaluate the effects of dependent variables, second-order polynomial model was used in the response surface analysis. For the explanation of RSM model, the following equation was used

$$Y = A_0 + \sum_{i=1}^n A_{ii}X_i^2 + \sum_{i=1}^n \sum_{j=i+1}^n A_{ij}X_i X_j$$

where X_i and X_j are independent variables, Y is the response, n is the number of variables ($n=4$), intercept A_0 is the offset term, A_i is the linear effect, A_{ii} is the squared effect, and A_{ij} is the interaction effect.

To run RSM for the selected sample of trail (T_{11}), because of its highest percentage yield, a mixture of maltose and lactic acid was used as DES. Here, the lactic acid acts as hydrogen bond donor and maltose act as

Table 1 Composition of various DES used for the extraction of chlorogenic acid

Trail no.	Mixtures of DES (1M H–D+ 1M H–A)	Trail no	Mixtures of DES (1M H–D+ 1M H–A)
T ₁	Water	T ₉	Acetamide and glycine
T ₂	Glucose and acetic acid	T ₁₀	Tartaric acid and glycine
T ₃	Glucose and tartaric acid	T ₁₁	Lactic acid and maltose
T ₄	Glucose and citric acid	T ₁₂	Glucose and lactic acid
T ₅	Fructose and citric acid	T ₁₃	Glucose and ascorbic acid
T ₆	Sucrose and citric acid	T ₁₄	Urea and sucrose
T ₇	Thiourea and glycine	T ₁₅	Acetic acid and Glucose+ Triton X 100
T ₈	D-Galactose and citric acid		

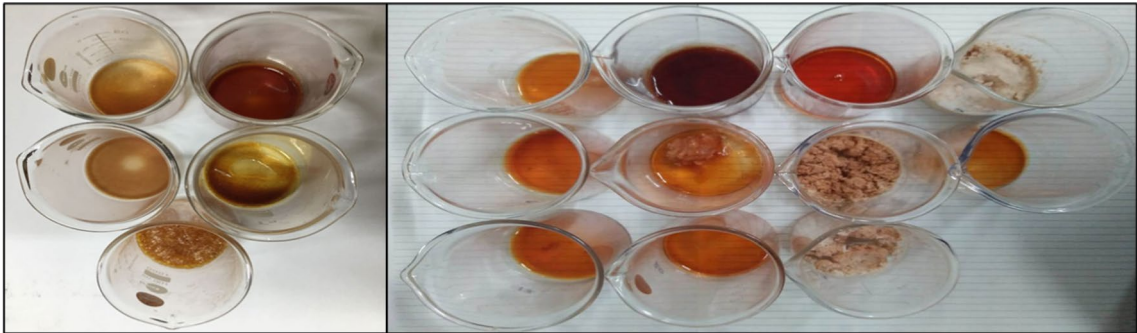


Fig. 3 Dried DES extracts in beakers

hydrogen bond acceptor. According to the set conditions, various combinations of X mL of 1 M lactic acid (L) and Y mL of 1 M maltose (M) were mixed well in a set of 21 Erlenmeyer flasks along with 1gm crushed powder of sea buckthorn. The flasks were placed on an orbital shaker for required time along with desired shaking speed as described in Supplementary Table 1. The contents of the flasks were filtered off with Whatman filter paper no. 1. The filtrates were placed in water bath at 60 °C in order to obtain viscous solutions which were then dried at room temperature. The experimental research and anticipated extraction values were calculated using Design Expert software version 10.2. The analysis of variance (ANOVA) and R^2 values were utilized to assess the model's significance and corresponding fitness.

HPLC analysis

The CGA in the plant extracts was quantified using HPLC. The extracts obtained by DES were subjected to RP-HPLC by using diode array detector (DAD) at 280 nm. Approximately 50 mg of each extract was refluxed with 20 mL of acidified methanol and 500 µg/mL butylated hydroxytoluene (BHT) as a preservative at 70 °C. The extracts were then chilled and centrifuged at 4000 rpm for 8 min. The trapped air in solutions was removed by sonication for 120 s. The solutions were filtered with the help of millipore (0.45 µm) filter paper. The resultant solutions were injected into the C-18 column of dimension of 5 µm particle size, L×(Internal diameter) I.D. 15 cm×4.6 mm one by one and were analyzed at 25 °C. The CGA in sea buckthorn was eluted and measured by employing a C-18 column. Mobile phase (A) contained water/acetic acid in ratio 94:6 and (B) acetonitrile (≥ 99.9%). For the elution of bioactive phenolic compounds, the flow rate was adjusted to 1 mL/min. The run time was 45 min, and the gradient mode was adjusted as 0 to 15 min 15% B, 15 to 30 min 45% B, 30 to 45 min 100% B [39, 40].

Total phenolic contents (TPC) assay

The determination of TPC was carried out spectrophotometrically by using Folin–Ciocalteu reagent (FCR). The method used for the determination of TPC was slightly modified as described earlier [41]. Standard stock solution of gallic acid (1000 µg/mL) was prepared, from which various concentrations of gallic acid (0, 25, 50, 75, 100, 125, 150, 175, and 200 µg/mL) were prepared. Then, 5 mL of 10% FCR and 4 mL of 7% Na_2CO_3 were added to each concentration making a total volume of 10 mL. The contents of the reaction mixtures were stirred and then incubated for 20 min at 40 °C in a water bath. Same procedure was repeated for extract in place of gallic acid. The dark blue color indicated that FCR had oxidized the

gallic acid and the phenols in the extract. The absorbance was measured at 765 nm, calibration curve was plotted from the absorbance of gallic acid concentrations, and TPC was calculated as milligram gallic acid equivalent per gram (mg GAE/g) from regression equation of calibration curve ($R^2=0.993$) [42, 43].

DPPH radical scavenging capacity (RSC) assay

In order to measure antioxidant activity and radical scavenging capacity of CGA from sea buckthorn extract, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl radical (DPPH) assay was performed. The stock solution was obtained by dissolving 1 mg/mL DPPH in 100 mL of methanol stored in dark. For negative control (blank), 2.5 mL stock solution was mixed with 100µL methanol similarly for sample 2.5 mL of methanolic solution of DPPH and 100µL of extract was added in two different cuvettes. Both the solutions were kept in dark for 30 min, and the absorbance was measured at 516 nm wavelength by using a UV–Vis spectrophotometer. The percentage of DPPH scavenging activity was determined based on following Eq. 2.

$$\text{DPPH scavenging activity \%} = \frac{[\text{Abs}(\text{blank}) - \text{Abs}(\text{sample})]}{\text{Abs}(\text{blank})} \times 100 \quad (2)$$

Abs (Blank)=negative control absorbance after half an hour. Abs (Sample)=absorbance of the extract solution after half an hour. The concentration of the extract (g/mL) (w/v) necessary to scavenge 50% of the free radical DPPH was used to express scavenging activity as IC_{50} . [44, 45].

Trolox equivalent antioxidant capacity (TEAC) assay

The ABTS radical was prepared according to previously described methods for TEAC assay [41, 46]. The ABTS radical cation ($\text{ABTS}^{+\bullet}$), a stock solution containing 7 mM ABTS, was prepared in 10 mL of 2.45 mM potassium hydrogen sulfate. After 24 h of dark incubation, the solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. In each experimental run, 100 mL sea buckthorn extract (5 mg/mL) was then incubated for 8 min with the same volume of a previously diluted $\text{ABTS}^{+\bullet}$ solution, and the absorbance was measured at 734 nm. As a positive control, trolox was used, and the antioxidant capacity was measured in micro-moles of trolox equivalents (µmol TE) per gram of sea buckthorn extract.

Trolox's absorbance at 734 nm was plotted to create a dosage response curve, and the percentage of each sample inhibited with time was computed using this formula

$$\text{Percentage Inhibition} = \left(1 - \frac{A_f}{A_o}\right) \times 100$$

where A_f is the absorbance after addition of sample and A_o is the absorbance of ABTS^{•+}.

Antioxidant activity in linoleic acid system

The antioxidant capacity of extracts was further analyzed by evaluating the percentage oxidation of linoleic acid method [46]. Approximately 5 mg extract was mixed with 130 μ L of linoleic acid solution in a 25 mL volumetric flask, and 10 mL of 99.8% ethanol and 10 mL buffer of 0.2 M, Na₃PO₄ with pH 7.0 were added and made to mark with distilled water and then incubated at 40 °C for 15 days. The degree of oxidation of the solution was measured by using thiocyanate method [46], in which 10 mL of 75% ethanol, 0.2 mL 30% NH₄SCN solution, 0.2 mL sample solution and 0.2 mL of 20 mM FeCl₂ solution in 3.5% HCl were added in sequence with 3 min of stirring. The peroxide contents were calculated by taking the absorption of mixtures at 500 nm. A positive control containing synthetic antioxidant butylated hydroxytoluene (BHT) in place of extract was also run. Equation 3 was used for the estimation of %age oxidation inhibition of linoleic acid [47].

$$\text{Percentage inhibition} = 100 - \left[\frac{\text{Abs of Sample at 360 h}}{\text{Abs of Control at 360 h}} \times 100 \right] \quad (3)$$

Statistical analysis

One-way analysis of variance (ANOVA) and Student's *t* test were used for statistics, and *P* value < 0.05 was considered statistically significant and represented by * asterisk. The software used was (Prism 8.0, GraphPad, SPSS version 22, and design expert software version 10.2). The values for mean and standard deviation were taken for at least 3 readings. The representation **P* < 0.05, ***P* < 0.01 is compared to control group. The data are presented as mean $\pm$ SD.

Results

Extraction efficacy

After carefully screening of the under-consideration plant material via various combinations of DES-based extraction, the Trails 6 and 11 gave the highest percentage yield 4.83% and 5.08% of CGA, respectively. The trial 11 was selected for further optimization and validation process. The reason of selecting this trail was relatively high percentage yield of the sample for CGA as shown in Fig. 4.

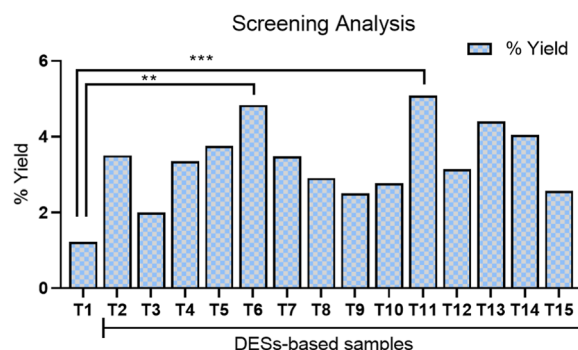


Fig. 4 Optimization of extraction yields using water and deep eutectic solvents (DES) mixtures

Quantitative and qualitative analysis of chlorogenic acid via HPLC

The quantitative estimation of CGA was done using high-performance liquid chromatography (HPLC). The chromatogram for retention time at corresponding peaks was demonstrated both for standard sample and plant extract for CGA in Fig. 5. The HPLC analysis of extracts obtained revealed significant presence of CGA as demonstrated by the retention time 4.12 ± 0.26 min at corresponding peak.

Optimization of parameters for chlorogenic acid extraction

The dry powder of sea buckthorn was treated with various compositions of DES for recovery of entrapped phenolic component CGA under various conditions of reaction parameters, e.g., shaking speed, shaking time, hydrogen bond donor/hydrogen bond acceptor (ml/ml) ratio, and liquid-to-solid (ml/g) ratio. The RSM was applied to get the maximum extraction yield of CGA from sea buckthorn under optimized conditions of reaction parameters with significant levels of reserved antioxidant activities as shown in Table 2.

The results appraised the correctness and quality of the model. Here, lactic acid was used as hydrogen bond donor (H-D) and maltose was used as hydrogen bond acceptor (H-Accep). The DES were used as mixture (L) and ground sample of sea buckthorn represented as (g). The study examined the maximum % yield under various parameters described earlier, e.g., (A) H-D/H-Accep, (B) L/S, (C) shaking time, (D) time. The factors investigated partially at different levels in a rotatable central composite. The experiment layout was subsequently expanded to factorial and axial points and response observed for statistical analysis using design expert software (version 10.2). Supplementary Table 1 describes the optimum conditions for extracting a good percentage yield of CGA. The optimized conditions for DES to get 12.4% yield were like hydrogen bond donor/hydrogen bond acceptor ratio

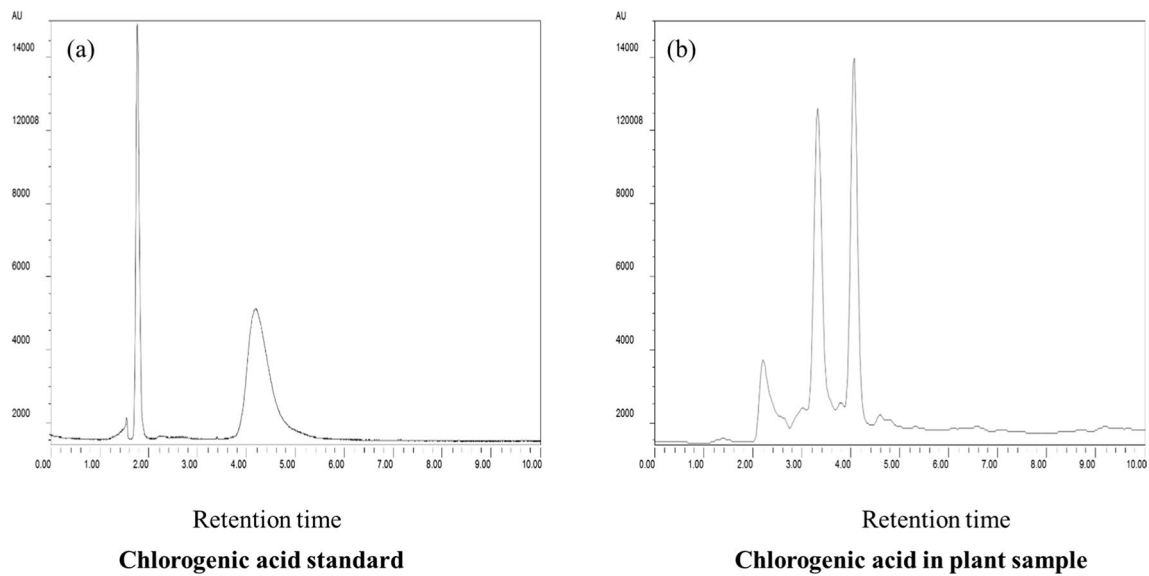


Fig. 5 HPLC chromatogram for (a) chlorogenic acid standard and (b) chlorogenic acid in plant sample

Table 2 The optimized RSM parameter for estimation of extractions yield

Source	Sum of Squares	Df	Mean Square	F value	P value
Model	170.03	14	12.15	159.88	< 0.0001
A:H-D/H-Accep	11.95	1	11.95	157.29	< 0.0001
B: L/S	5.17	1	5.17	68.08	0.0002
C-Shaking speed	0.0022	1	0.0022	0.0291	0.8701
D-time	9.38	1	9.38	123.46	< 0.0001
AB	3.57	1	3.57	46.99	0.0005
AC	3.79	1	3.79	49.92	0.0004
AD	0.1190	1	0.1190	1.57	0.2574
BC	1.26	1	1.26	16.60	0.0065
BD	4.81	1	4.81	63.29	0.0002
CD	0.4560	1	0.4560	6.00	0.0498
A ²	69.12	1	69.12	909.93	< 0.0001
B ²	0.0042	1	0.0042	0.0559	0.8209
C ²	6.07	1	6.07	79.87	0.0001
D ²	0.5951	1	0.5951	7.83	0.0312
Residual	0.0021	6	0.0004		
Lack OF Fit	0.0000	2	0.0000	0.0351	0.9658
Pure error	0.0021	4	0.0005		
Cor total	13.84	20			

1.35, liquid-to-solid ratio 1.00, shaking speed 100 rpm, and shaking time 60 min [48].

After recovery of phenolic antioxidant, CGA under various reaction parameter conditions of shaking speed, shaking time, hydrogen bond donor/hydrogen bond

acceptor (ml/ml) ratio, liquid-to-solid (ml/g) ratio, the analysis of extraction yield was carried out. The model F value 159.88 with probability $P < 0.0001$ implies that the model is significant and there are only 0.001% chances that large F value could occur due to noise. Parameters having values of probability P less than < 0.0500 indicate model terms are significant. The variable A: H-D/H-Accep, B: L/S, C: shaking speed, D: shaking time, interactions AB, AC, BS, CD, and quadratic effects A^2 , B^2 , C^2 , D^2 were adjusted by their corresponding F-ratio and probability P values. Similarly, “Lack of fit F value” of 0.00 implies that model selected has good fit.

The reliability and reproducibility of observed results were invariants by observed coefficient of variation ($CV = 3.11\%$). Hence, second-order polynomial regression equation can be successfully used to plot out the response. In this context, a typical second-order polynomial equation for phenolic extracts, producing maximum amount of phenolic component CGA, among all DES can be deduced with the help of following equation.

$$Y = + 10.85 + 2.44A + 1.61B + 0.0149C + 2.17D + 1.49AB - 0.6885AC + 0.2727AD - 0.3970BC + 1.73BD + 0.2388CD - 5.20 A^2 - 0.0408 B^2 + 1.54 C^2 - 0.4828 D^2$$

where the extraction yield of antioxidant component CGA is symbolized by Y. The second-order polynomial equation illustrates the linear effects, interaction, and quadratic terms which are ultimate for the extraction yield. The insignificant factors should remove from the fitted second-order polynomial equation which is

mentioned above, and fitted second-order polynomial regression equation is given below as.

$$Y = +10.85 + 2.44A + 1.61B + 2.17D + 1.49AB - 0.6885AC - 0.3970BC + 1.73BD + 0.2388CD - 5.20 A^2 + 1.54 C^2 - 0.4828 D^2$$

Estimation of antioxidant potential by quadratic polynomial expression

A further extraction of antioxidants CGA under particular reaction parameters was presented by quadratic polynomial expression when value of R^2 (0.9973) is higher and presented with help of quadratic polynomial expression. Similarly, the adjusted R^2 value validated the good agreement between actual and observed extraction yield. The interaction can be predicted with the help of three-dimensional quadratic profiles for yield of phenolic acid component CGA shown in the following graphs of Fig. 6.

Figure 6 represents the prominent effect of hydrogen bond donor to hydrogen bond acceptor on different reaction parameters like liquid-to-solid ratio, shaking speed, and shaking time. The best percentage yield extraction for phenolic component CGA at optimized reaction parameters of L/S ratio was 4, and shaking speed of 175 rpm and shaking time of 90 min were found after careful analysis.

A liquid-to-solid ratio below 3 results in sharp decrease in extraction yield, and above 3 results have no prominent impact in extraction yield, whereas in range of factor A: 1.3–1.6 results in sharp increase in extraction yield. As further moving from 1.6, the extraction yield started to decrease. The value for factor A: 1.5 was found to be the most favorable point for extraction of phenolic antioxidant CGA.

The results help to interpret the interaction between shaking speed and hydrogen bond donor to hydrogen bond acceptor. The data given in Fig. 6 indicate maximum

yield within 1.3–1.6 of factor A. These ratio points yield gone up to 9%. However, the effect of shaking speed is

not dominant. Factor A has no more influential outcome on extraction yield from 1.6–2.0 ratio.

The results also elucidate the relationship between shaking time and factor A. The shaking time has no influential impact on overall extraction yield in regard to variable factor A values. The matrix liberated antioxidant by medium of DES from 1.3 to 1.6 ratio of factor A exceeds the percentage yield from 9% of extraction. However, further increase in factor A above 1.6 ratio will lead to a decrease in overall extraction yield.

Impact of interaction among reaction parameters on extraction output

Three-dimensional RSM was employed to evaluate the effect of reaction parameters interrelationship on overall extraction yield. The results schemes in Fig. 7 elucidated the relationship between shaking speed and liquid-to-solid ratio. The graph depicted that liquid-to-solid ratio and shaking speed have no significant effects on overall extraction yield of CGA. The curve in this form revealed that both factors have no remarkable effect on the percentage of yield of phenolic component CGA.

The three-dimensional graphs show above the interactions between liquid-to-solid ratio and shaking speed and time parameters affecting the overall extraction. The results signify that shaking time has prominent impact on percentage extraction of CGA. As the time increases from 90 to 120 min, the yield obtained increases from

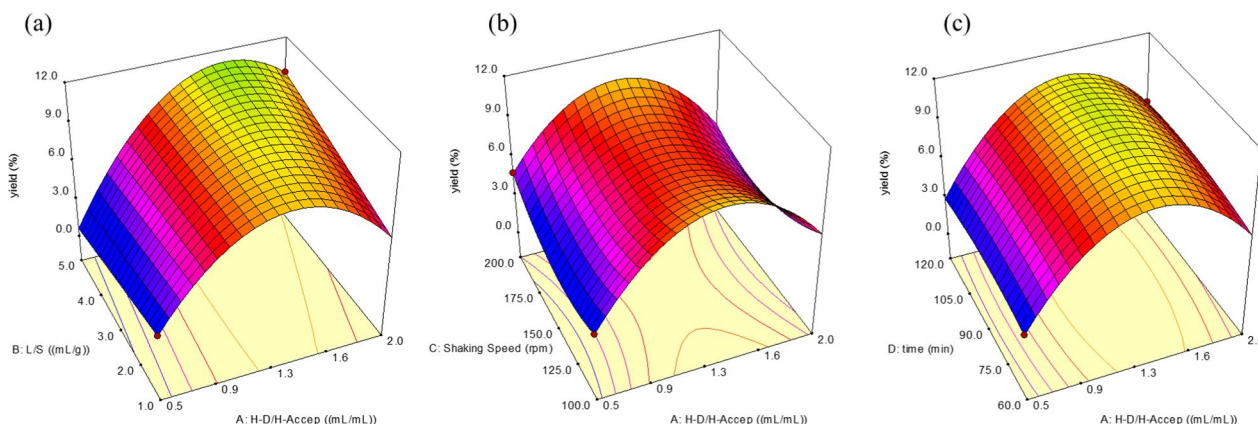


Fig. 6 Three-dimensional surface response analysis of the relationships for extraction percentage of CGA (a) liquid–solid ratio and H–D-to-H-Accep ratio, (b) shaking speed and H–D-to-H-Accep ratio, and (c) extraction time and H–D-to-H-Accep ratio

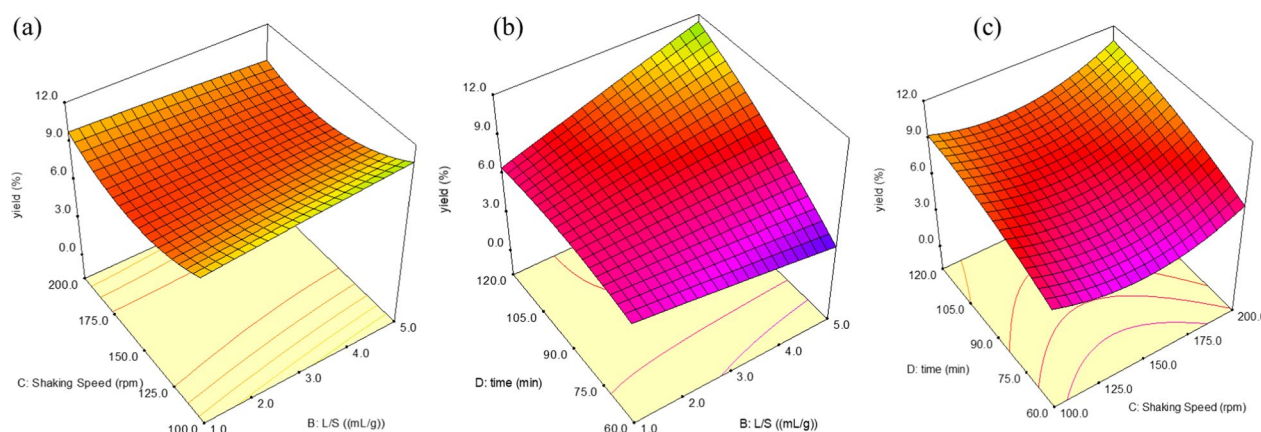


Fig. 7 Three-dimensional surface response of the interrelationships for CGA extraction (a) shaking speed and liquid–solid ratio, (b) extraction time and liquid–solid ratio, and (c) extraction time and shaking speed

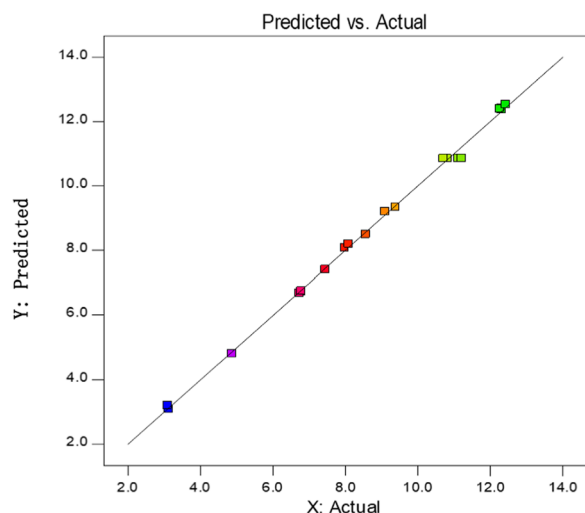


Fig. 8 Actual versus predicted values for extraction yield

6% to nearly 12%. The factor D has prominent impact on shaking speed as well. The shaking speed at 175 rpm for 120 min will induce the extract to liberate more phenolic antioxidant CGA. Overall, the time from 90 to 120 min provides good extraction yield of CGA.

Procedure robustness analysis

The association between predicted and actual extraction values illustrated with the help of straight-line graph which gave both robustness of the straight-line plot and validity of the model elucidated from fitted model and results of ANOVA gave the concordant explanation as shown in Fig. 8.

This straight-line graph explained the extraction yield from the fitted model was in the line that decided the actual values under optimum experimental conditions, with elevated coefficient of determination $R^2=0.9973$.

The following parameters describe the optimum conditions for extracting a good percentage yield of phenolic component CGA. The optimized condition for DES to get ~12% yield is such that hydrogen bond donor-to-hydrogen bond acceptor ratio 1.35, liquid-to-solid ratio 1.00, shaking speed 100 rpm, and shaking time 60 min are depicted in Fig. 9.

Activity analysis of CGA extracted under optimized conditions

Total phenolic content (TPC) analysis, radical scavenging capacity evaluation, linoleic acid assay, and trolox equivalent antioxidant capacity (TEAC) assay were performed to evaluate activity of extracted CGA. Folin–Ciocalteu reagent (FCR) was used for the determination of TPC of DES-based extraction of antioxidant CGA, which measures the sample's reducing capacity. A standard curve using different concentrations of gallic acid (50–200 ppm) ($R^2=0.6595$) was drawn from which the concentration of phenols in the test samples was calculated. The results were expressed as gallic acid equivalent (GAE) per gm dry matter. Total phenolic content in extract was found to be of maximum value 175 mg GAE/100 mg at most suitable conditions of shaking time 60 min, shaking speed 100 rpm, liquid/solid ratio 1.00, and hydrogen bond donor/hydrogen bond acceptor in 1.35 ratio as shown in Table 4. This indicated that applied DES have effectively broken plant cell wall and increased the recovery of plant phenolics content CGA.

DPPH scavenging capacity of extracted CGA was also evaluated. The DPPH scavenging radical has ability to accept an electron or hydrogen radical to become a stable diamagnetic molecule with different colors. The extracted CGA obtained under optimum conditions exhibited significant DPPH scavenging ability (≥ 90) indicates that

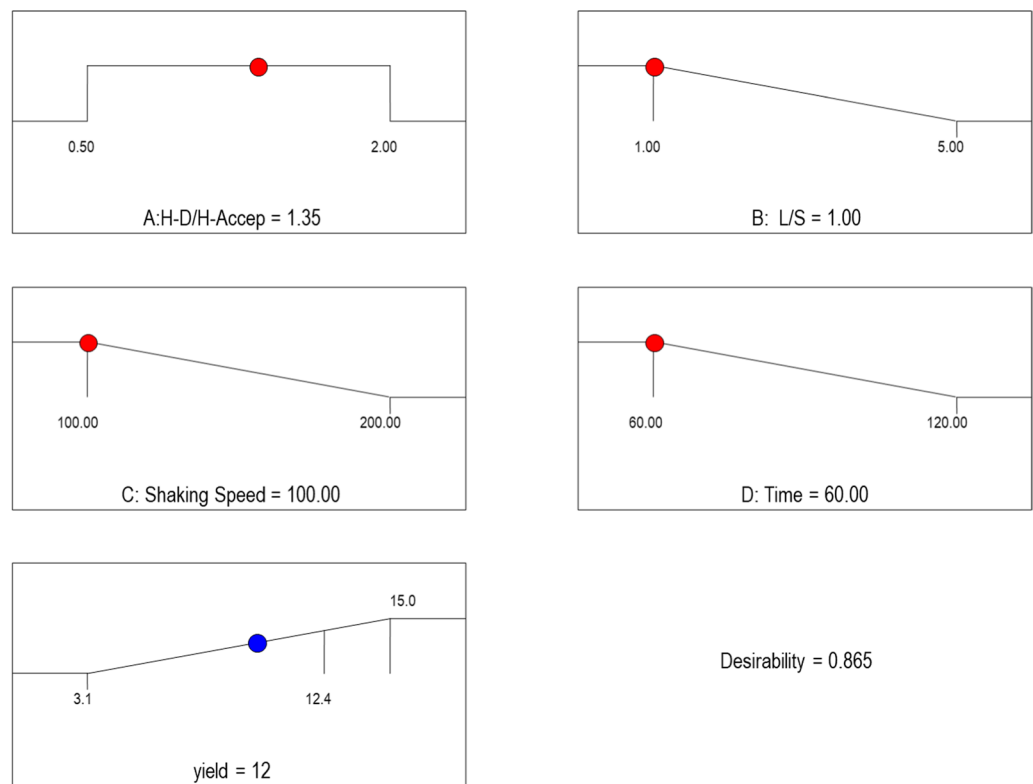


Fig. 9 Optimized condition of reaction parameters to get maximum percentage yield from combination of different DES parameters

Table 4 The extraction yield of CGA obtained under optimized conditions of reaction parameters

Sr No.	Optimized conditions				Response observed					
	H–D/H-Accep	(L/S)	Shaking speed (rpm)	Shaking time (min)	%age Yield	L.A	TPC	TEAC	DPPH	CGA (mg/g of extract)
01	1.35	1.00	100	60	12.30	80.5	174.5	112	92	20.1
02	1.35	1.00	100	60	12.10	80.5	174.7	112	91	20.1
03	1.35	1.00	100	60	12.20	81.0	175.0	112	90	20.1
Mean values					12.20	80.6	174.7	112	91	20.1

the DES have significantly improved the liberation of this phenolic compound without deteriorating the free radical scavenging efficacy (Table 4).

The evaluation of oxidation inhibition in a lipid-based system is another important assay to investigate antioxidant potential of plant extract. Generally, linoleic acid is used for the estimation of percentage inhibition of peroxidation. The oxidation inhibition potential was determined as 81% of linoleic acid by sea buckthorn extract (Table 4).

The cation radical of ABTS trapped the free radicals from the sea buckthorn extract and assessed with the help of spectrophotometer at 734 nm. The

antioxidant potential of CGA was maximum for the extract obtained under optimum conditions of reaction parameter.

Hydrogen bond donor to hydrogen bond acceptor (H–D/H-Accept), liquid-to-solid ratio (L/S), shaking speed and time, percentage yield, linoleic acid (L.A), total phenolic contents (TPC), trolox equivalent antioxidant capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and chlorogenic acid (CGA) extract are the values in gm/100 gm, mg/ml (IC₅₀), mg GAE/gm, TE/g, and mg/g, respectively.

Discussion

The primary objective of this investigation was to optimize and validate the extraction process of CGA from powdered sea buckthorn berries utilizing DES system. The combination of DES imparts specific characteristics to the resultant mixtures, designating them as green and environmentally friendly solvent class. [49, 50]. Notably, ethyl lactate, derived from corn processing, best exemplifies as green solvent [51, 52].

Recent revelations indicating the superior aptitude of DES to dissolve phenolic components prompted the design of current study for extraction of CGA from sea buckthorn [3, 53]. Initial experiments involved the preparation of DES through diverse combinations, subsequently treating sea buckthorn powder with these DES. Following a screening process, trial 11 (T11), featuring lactic acid as an HBD and maltose as an HBA, was identified for further optimization due to its highest extraction yield of 5.08% CGA.

The optimization process, employing response surface methodology, encompassed varied reaction parameters, including shaking speed, shaking time, hydrogen bond donor/hydrogen bond acceptor ratio, and liquid-to-solid ratio. A rotatable central composite design, modified with factorial and axial points, facilitated the analysis of response variations, with statistical significance set at $P \leq 0.05$.

The model's high F value (159.88) and low probability ($P < 0.0001$) indicated its significance, corroborating the importance of A: H-D/H-Accep, B: L/S, C: shaking speed, and D: shaking time, along with their interactions and quadratic effects. Furthermore, a lack of fit F value of 0.00 affirmed the model's fit, ensuring the consistency and repeatability of results (observed coefficient of variation: 3.11%). The second-order polynomial regression equation accurately depicted the phenolic acid extraction under optimal conditions.

Extrapolating from quadratic polynomial expressions, notably with a high R^2 value of 0.9973, and validated adjusted R^2 values, the study demonstrated a strong agreement between actual and observed extraction yields. The application of 3D response surface graphs effectively visualized the interrelation among variables.

DES, as mixtures of HBD and HBA compounds, form eutectic systems with lower melting points ($< 100^\circ\text{C}$), making them preferable solvents due to the interaction of van der Waals forces and hydrogen bonds [54, 55]. The molar ratio of lactic acid and maltose (1:1) with an optimum volume ratio of 1.35 enhanced stability and extraction capability, attributing to strong hydrogen bonding [56, 57].

The qualitative and quantitative estimation of CGA, facilitated by HPLC, under optimum conditions,

confirmed significant existence of CGA in the sea buckthorn extract. Additionally, the antioxidant potential was assessed through various methods, with promising results, including total phenolic content, oxidation inhibition, and CGA yield.

The application of DPPH free radical scavenging assays further highlighted the enhanced antioxidant potential of the sea buckthorn extract, with a low IC₅₀ (mg/ml) observed under optimal reaction parameters. Notably, FCR assay supported the enhanced extraction yield of CGA by DES, ensuring biosafety and efficacy.

The study highlights substantial improvement of CGA extraction yield from sea buckthorn using DES. The findings not only contribute to the novel application of DES in phenolic component extraction but also suggest potential extensions of this methodology for extracting various bioactive compounds from natural sources.

Conclusions

The novel utilization of DES for the extraction of the phenolic component chlorogenic acid from sea buckthorn berries has resulted in a significant enrichment in extraction yield. This innovative approach not only demonstrates improved productivity but also addresses the broader environmental concerns associated with the conventional use of petrochemical solvents for the same purpose. The sustainable extraction achieved through DES not only upholds a green and environmentally friendly ethos but also ensures economic viability, exceptional extraction adaptability, and evidently lower toxicity compared to traditional organic solvents. This shift toward DES-based extraction methods aligns with the overarching objective of minimizing environmental pollution.

Importantly, the antioxidant potential of the extracted material remains intact following DES-based extraction, affirming the suitability of this method for obtaining bioactive compounds while preserving their inherent properties. The inherent advantages of DES, including its reduced environmental impact and heightened biosafety, position it as a promising alternative for future extraction processes. The adaptability and versatility demonstrated by this methodology, especially under optimized conditions, make it a robust approach for determining the antioxidant capacity of phenolic antioxidants. However, scalability and practicality of this innovative approach remains a challenge until pilot scale-up studies will be carried out.

Looking ahead, the current methodology holds promise for broader applications with slight modifications, potentially extending its utility to extract various other bioactive compounds. The findings presented herein underscore the transformative potential of DES in the realm of extraction processes, laying the groundwork for

the development of sustainable, efficient, and environmentally conscious methodologies in the field of natural product extraction.

Abbreviations

ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CGA	Chlorogenic acid
DAD	Diode array detector
DES	Deep eutectic solvents
DPPH	2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl
FCR	Folin–Ciocalteu reagent
GA	Gallic acid
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HPLC	High-pressure liquid chromatography
ILs	Ionic liquids
RSC	Radical scavenging capacity
RSM	Response surface methodology
TEAC	Trolox equivalent antioxidant capacity
TPC	Total phenolic contents
UV	Ultraviolet

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-024-00699-x>.

Additional file 1.

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Author contributions

M.M. contributed to conceptualization, I.S. and A.S. were involved in methodology, S.R. provided software, M.A.G. contributed to validation and data curation, M.M., M.A.G. and S.R. were involved in formal analysis, I.S. contributed to investigation and resources, A.S., S.R. and M.A.G. were involved in writing—original draft preparation, and visualization, and I.S., A.S., M.A.G. and A.R. contributed to writing—review and editing. A.S., M.M. and M.A.G. contributed to supervision, and M.M. was involved in project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.”

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Availability of data and materials

Data and material are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Plant authentication

Sea buckthorn berry was purchased from a local market in Gilgit-Baltistan. The specimen of the plant part was identified as *Hippophae rhamnoides* by taxonomist at GCU Lahore, and a sample was also submitted there in the herbarium with voucher number 6875F/Bot. Moreover, the plant name has also been checked with <http://www.theplantlist.org> for the accepted name in accordance with the International Plant Names Index (IPNI).

Consent for publication

Not applicable.

Competing interests

Authors declare no competing interest.

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