

Green Extraction and Optimization of Bioactive Compounds from Elderberry (*Sambucus nigra* L.) by β -Cyclodextrin Assisted Extraction Short title: Optimized Green Extraction of Elderberry Bioactives with β -Cyclodextrin

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Abstract

Phenolics of elderberry (*Sambucus nigra* L.) were recovered via a green, β -cyclodextrin (β -CD)–assisted aqueous extraction. Process effects were mapped using response surface methodology (face-centered central composite design) with β -CD 1–3% (w/v), liquid–solid (L/S) ratio 10–30% (w/v), and time 30–90 min. Responses (TPC, TFC, TMAC, TPAC; DPPH, FRAP, CUPRAC) were modeled to identify operating windows. TPC followed a quadratic relationship; TFC a reduced quadratic with an optimum near $\sim$ 2% β -CD, whereas TMAC and TPAC exhibited approximately linear trends across the studied space. DPPH showed clearer parameter sensitivity than FRAP/CUPRAC, and extraction time exerted a secondary effect within 30–90 min. Contour plots consistently indicated low-to-moderate L/S together with $\sim$ 2% β -CD as a region that balances multiple responses while avoiding dilution or mass-transfer limitations. Overall, β -CD–assisted extraction provides an effective, solvent-lean route for recovering elderberry phenolics and delineates a practical window for greener processing. These results motivate follow-up work on inclusion mechanisms, stability during storage and digestion, and scale-up supported by techno-economic and life-cycle assessments.

1. Introduction

Phenolic compounds, a class of secondary metabolites including pigments, have long garnered significant attention due to their diverse functional properties. These compounds play a crucial role in determining the organoleptic characteristics—such as taste, color, and aroma—of plant-based foods and are abundantly present in fruits and vegetables. In particular, phenolics are largely responsible for the characteristic coloration observed in many fruit and vegetable species, contributing both to their visual appeal and potential health benefits ¹.

Elderberry (*Sambucus nigra* L.) is a rich source of bioactive compounds, particularly polyphenols, and is well known for its strong antioxidant capacity ^{2–5}. Among its notable constituents are anthocyanins, which contribute not only to its health-promoting properties but also to its potential use as a natural colorant. Elderberry powders and extracts have been successfully incorporated into various food products for coloring purposes ^{6–8}. Owing to these functional and technological attributes, elderberry is increasingly utilized as a functional ingredient and natural additive in the development of health-oriented food products ⁹(MARTIS).

β -CDs are cyclic oligosaccharides produced through the enzymatic degradation of starch. They consist of six, seven, or eight D-(+)-glucopyranose units linked by α -1,4 glycosidic bonds ¹⁰. Among natural cyclodextrins, β -CD is the most accessible, cost-effective, and widely utilized form ¹¹. Recognized as Generally Regarded As Safe (GRAS), β -CD is extensively used in the food industry due to its ability to form inclusion complexes with various bioactive compounds, thereby enhancing their stability, solubility, and bioavailability ¹². When employed as an extraction medium in aqueous systems, β -CD facilitates the effective recovery of bioactive compounds from plant matrices by interacting with target molecules ¹³. Previous studies have demonstrated the successful application of β -CD in improving the extraction efficiency of bioactives from various plant sources, including red beetroot ¹⁴, olive and red grape pomace ^{15,16}, whole pomegranate fruit ¹⁷, and peach pomace ¹⁸.

In this study, phytochemicals were extracted from elderberry using β -CD as a green solvent, and the bioactive properties of the resulting extracts were evaluated. The effects of key extraction parameters—namely β -CD concentration, liquid-to-solid ratio, and extraction time—on the recovery of phenolics, antioxidants, flavonoids, anthocyanidins, and proanthocyanidins were systematically examined using a Central Composite Design (CCD). This experimental approach enabled the identification of optimal extraction conditions for maximizing the yield of bioactive compounds from elderberry.

2. Materials and methods

2.1. Materials

Approximately 3 kg of elderberries (*Sambucus nigra* L.) were procured from a local market in Konya, Turkey. The fruits were thoroughly washed and cleaned to remove any foreign materials. Drying was performed using a vacuum oven (Eksis Makine, Isparta, Turkey) at 50°C. The dried elderberries were then ground into a fine powder using a laboratory blender. The resulting elderberry powder was stored in sealed polyethylene bags at -20°C until further use. β -CD was obtained from Sigma-Aldrich (St. Louis, MO, USA), while all other analytical-grade chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Extraction

Extraction was performed following Kim, Son, Pang, Yang, Lee, Lee, Lee, Park and Yoo ¹⁹, with modifications. Based on the experimental design, predetermined amounts of elderberry powder were accurately weighed into extraction vessels. Corresponding volumes of β -CD solutions were added to the samples according to the design parameters. The mixtures were homogenized at 10,000 rpm for 1 min using an Ultraturrax homogenizer (IKA T25 digital, Staufen im Breisgau, Germany). The samples were placed in a shaking water bath (Nüve, NB-20, Ankara, Türkiye) (60°C , 70 rpm) for 1 h. Following homogenization, the samples were centrifuged at $10,000 \times g$ for 15 min (Hermle Z326K, Germany). The resulting supernatants were filtered (Saha, Turkey) to remove particulates and subsequently stored at $+4^{\circ}\text{C}$ until further analysis.

2.3. Experimental design

In this study, a face-centered CCD was employed to evaluate the effects of three independent variables on the extraction efficiency of bioactive compounds from elderberries. The selected factors and their levels were as follows: β -CD concentration (1%, 2%, 3%) ^{18,20}, liquid-to-solid ratio (10%, 20%, 30%) ²¹, and extraction time (30, 60, and 90 min) ^{22,23}. As presented in Table 1, the design consisted of 15 experimental runs, including three replicates at the center point to estimate experimental error. 7 response variables were measured for each extract: TPC, antioxidant activity (DPPH, FRAP, and CUPRAC assays), TFC, TMAC, and TPAC.

Mathematical models for each response were developed using least squares regression based on the experimental data. The statistical significance of model terms was evaluated at a 90% confidence level using p-values. Analysis of variance (ANOVA), lack-of-fit testing, and determination of coefficients (R^2) were performed using Design-Expert Version 10 software (Stat-Ease Inc., Minneapolis, USA). A desirability function approach was applied to determine the optimal extraction conditions by simultaneously maximizing all response variables. No additional experimental validation of the model was conducted.

Table 1
Experimental design and responses of elderberry extracts

Std order	Run order	β -cyclodextrin concentration (%)	Liquid-solid ratio (% w/v)	Extraction time (min)	TPC (g GAE/kg dm)	DPPH (g TE/kg dm)	FRAP (g TE/kg dm)	CUPRAC (g TE/kg dm)	TFC (mg CE/kg dm)	TMAC (mg C3G/kg dm)	TPAC (g cyanidin/kg dm)
10	1	2	30	30	51.03	98.04	50.89	48.79	18189	9225	17.17
12	2	2	30	90	51.96	98.04	85.86	75.24	20521	8652	26.20
15	3	2	20	60	47.59	74.81	71.01	59.82	19454	8620	22.67
3	4	1	30	60	46.71	85.62	66.89	68.37	16773	7800	23.98
5	5	1	20	30	44.59	83.09	80.80	54.34	16197	7399	23.26
11	6	2	10	90	44.34	84.76	75.10	66.32	20009	8732	25.75
4	7	3	30	60	45.00	78.47	100.19	65.32	14497	7897	23.21
9	8	2	10	30	38.43	78.64	97.30	72.48	19898	8359	24.97
13	9	2	20	60	46.97	68.87	90.72	64.85	18418	9430	24.89
6	10	3	20	30	46.61	73.37	67.53	64.51	19380	8890	19.75
2	11	3	10	60	37.00	54.16	82.04	55.58	18862	9701	24.32
7	12	1	20	90	49.67	74.27	84.03	50.95	17048	8042	25.40
1	13	1	10	60	43.30	76.56	73.61	47.62	18656	9058	20.72
14	14	2	20	60	48.42	85.43	98.66	63.44	18362	8480	27.58
8	15	3	20	90	45.08	59.78	65.67	49.82	17085	8182	24.04

2.4.Extraction analyses

2.4.1.Determination of TPC of extracts

The TPC of the elderberry extracts was determined using the Folin–Ciocalteu colorimetric method, as described by Georgiev, Weber, Kneschke, Denev, Bley and Pavlov ²⁴, with slight modifications. Briefly, each extract was appropriately diluted with distilled water. Then, 0.2 mL of the diluted sample was mixed with 2.0 mL of 0.2 N Folin–Ciocalteu reagent, followed by the addition of 0.8 mL sodium carbonate solution (75 g/L, Na_2CO_3). The mixture was incubated for 30 min at room temperature in the dark. After incubation, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Biochrom Libra S22, Cambridge, UK). Results were expressed as grams of gallic acid equivalents per kilogram of dry matter (g GAE/kg dm).

2.4.2.Determination of antiradical activity of extracts

The antioxidant capacity of elderberry extracts was evaluated using three widely applied assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Cupric Ion Reducing Antioxidant Capacity (CUPRAC). The DPPH radical scavenging activity was determined with slight modifications based on the method of Tontul and Topuz ²⁵. Initially, the elderberry extracts were diluted with distilled water. Then, 950 μL of freshly prepared DPPH solution (60 μM in methanol) was added to 50 μL of the diluted extract. The mixture was incubated for 30 min at room temperature in the dark. Absorbance was measured at 516 nm using a UV–Vis spectrophotometer. The FRAP assay was carried out according to the method described by Granato, Karnopp and van Ruth ²⁶Granato et al. (2015), with minor modifications. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM ferric chloride hexahydrate in a 10:1:1 (v/v/v) ratio. After pre-incubation of the FRAP reagent at 37°C for 10 min, 100 μL of each extract was added. The mixture was incubated in the dark at 37°C for 30 min, and absorbance was measured at 593 nm. CUPRAC analysis was performed following the procedure described by Apak, Güçlü, Özyürek and Karademir ²⁷. The reaction mixture was prepared by combining equal volumes (1:1:1, v/v/v) of 7.5 mM neocuproine, 10 mM copper(II) chloride (CuCl_2), and 1 M ammonium acetate buffer (pH 7.0). To this mixture, 0.5 mL of the extract and 0.6 mL of distilled water were added. The final mixture was incubated at room temperature for 30 min in the dark. Absorbance was recorded at 450 nm. A blank was prepared by replacing the sample volume with an equivalent volume of the reaction mixture. All antioxidant activity results were expressed as grams of Trolox equivalents per kilogram of dry matter (g TE/kg dm).

2.4.3.Determination of TFC of extracts

The TFC of the elderberry extracts was determined using the colorimetric method described by Chang, Lin, Chang and Liu ²⁸, with slight modifications. Briefly, 0.5 mL of the extract was mixed with 2.5 mL of distilled water and 0.15 mL of 5% sodium nitrite (NaNO₂) solution. The mixture was allowed to stand for 5 min at room temperature. Subsequently, 0.3 mL of 10% aluminum chloride (AlCl₃) solution was added, and the reaction was allowed to proceed for an additional 5 min. Then, 1.0 mL of 1 M sodium hydroxide (NaOH) was added to the mixture, which was then vortexed vigorously. The absorbance was measured at 510 nm against a distilled water blank using a UV–Vis spectrophotometer. TFC was expressed as grams of catechin equivalents per kilogram of dry matter (g CE/kg dm).

2.4.4.Determination of Total Monomeric Anthocyanin Content of extracts

The TMAC of the elderberry extracts was determined using the pH differential method, as described by ²⁵, with slight modifications. The extracts were appropriately diluted with buffer solutions at pH 1.0 and pH 4.5. The diluted samples were allowed to equilibrate in the dark for 20 min at room temperature. Absorbance readings were then taken at 510 nm (λ_{max}) and 700 nm against a distilled water blank using a UV–Vis spectrophotometer. The anthocyanin content was calculated and expressed as grams of cyanidin-3-glucoside equivalents per kilogram of dry matter (g C3G/kg dm).

2.4.5.Determination of Total Proanthocyanin Content of extracts

The TPAC of the elderberry extracts was determined following the method described by Eyiz, Tontul and Turker ²³, with slight modifications. A solution of FeSO₄·7H₂O (154 ppm) was prepared in a mixture of hydrochloric acid and *n*-butanol (2:3, v/v). In a stoppered glass tube, 2.5 mL of this reagent was added to 0.25 mL of the extract. The mixture was then incubated in a water bath at 95°C for 15 min. After incubation, the absorbance was measured at 540 nm using a UV–Vis spectrophotometer. Results were expressed as grams of proanthocyanidins per kilogram of dry matter (g TPAC/kg dm).

2.5. Statistical analysis

All data were expressed as mean values $\pm$ standard deviations based on triplicate measurements. Statistical analyses were performed using one-way analysis of variance (ANOVA) to determine significant differences among experimental groups, and Pearson correlation analysis was applied to assess relationships between the measured variables. ANOVA results were considered statistically significant at $p < 0.05$, while Pearson correlation coefficients were evaluated at significance levels of $p < 0.05$ and $p < 0.01$.

In addition, Response Surface Methodology (RSM) based on a face-centered CCD was employed to model and optimize the effects of independent variables— β -CD concentration, liquid-to-solid ratio, and extraction time—on the recovery of bioactive compounds. Design-Expert version 10 software (Stat-Ease Inc., Minneapolis, USA) was used for regression analysis, analysis of variance (ANOVA), lack-of-fit testing, and optimization through desirability function.

3. Results and discussions

3.1.TPC

Extracting natural bioactives like polyphenols is often a time-consuming process that uses organic solvents. This can be bad for the environment and limit how useful they are in practice. Furthermore, harsh extraction conditions can result in the degradation of the compound, thereby reducing its bioavailability ²⁹. TPC values of elderberry extracts ranged from 37.00 to 51.96 g GAE/kg dm, with an average of 45.78 g GAE/kg dm and a coefficient of variation of 8.97% (Table 1). Furthermore, TPC values of elderberry extract samples are illustrated in Fig. 1a. 2% β -CD, 30% liquid-to-solid ratio, 90 min had the highest TPC, while 3% β -CD, 10% liquid-to-solid ratio, 60 min had the lowest (Fig. 1a). According to the RSM analysis (see Table 2), the TPC response was best described by a quadratic model ($R^2 = 0.9330$; Adj $R^2 = 0.8124$). At a fixed liquid-to-solid ratio of 20 and an extraction time of 30 min, increasing the concentration of β -CD did not notably enhance TPC. Similar trends were observed in phenolic yield studies with mint ³⁰. Both β -CD concentration (A^2) and the liquid-to-solid ratio (B^2) exhibited significant negative quadratic effects ($p \approx 0.05$). Figure 3a clearly illustrates the combined influence of β -CD concentration and liquid-to-solid ratio on TPC. The orange–yellow regions indicate the highest TPC values (~ 48 – 50 g GAE/kg dm), corresponding to a moderate β -CD concentration (2%) and a medium–high liquid-to-solid ratio (20–25%). The green and blue zones represent lower TPC levels, with the minimum occurring at a high β -CD concentration (3%) and a low liquid-to-solid ratio (10%).

Phenolic compounds are generally highly soluble in water due to their ability to form hydrogen bonds. However, the relatively low water solubility of β -CD ³¹ and the presence of a secondary hydrogen-bonding network within its structure that limits interactions with water molecules ^{32, 33} can reduce mass transfer at high β -CD levels. Additionally, β -CD presents a hydrophobic inner surface. Therefore, it is better for compounds with high polarity instead ³⁴. Consequently, as the concentration of β -CD increases, the extraction yield of water-soluble phenolic compounds may decrease.

In this study, the liquid-to-solid ratio also negatively affected TPC, suggesting that exceeding the optimum level could cause dilution of phenolic compounds in the solvent phase and reduce extraction efficiency. In contrast, studies on whole pomegranates have reported increases in TPC at higher β -CD levels ¹⁷. Such discrepancies are likely due to differences in the plant matrix, extraction methods and liquid-to-solid ratios applied.

3.2.Antioxidant activity analyses (DPPH, FRAP, CUPRAC)

Changes in DPPH, FRAP, and CUPRAC antioxidant activities of elderberry extract samples are presented in Table 1 and Figs. 1b, 1c, and 1d. The results showed that antioxidant activities, measured by the DPPH, FRAP, and CUPRAC methods, ranged from 54.16 to 98.04, 50.89 to 100.19, and 47.62 to 75.24 g TE/kg dm, respectively. The highest DPPH activity was observed in the sample, extracted using 2% (w/v) β -CD concentration, 30% liquid-solid ratio, and 90 min extraction time. This same sample exhibited the highest antioxidant activity according to CUPRAC assays (Table 1). On the other hand, the maximum FRAP activities were detected in the sample, prepared with 3% (w/v) β -CD concentration, 30% liquid-solid ratio, and 60 min extraction time.

According to two-factorial ANOVA, the interaction between β -CD concentration and liquid-solid ratio significantly influenced the antioxidant activities, and Reduced Quadratic was identified as the most suitable. Although the TPC yield was best described by a quadratic model, the DPPH radical scavenging activity was more accurately represented by a reduced quadratic model. A positive correlation between TPC and DPPH values is commonly observed in most plant extracts. Nevertheless, deviations from this pattern have been reported, often attributed to synergistic or antagonistic interactions among the bioactive constituents within the extracts^{35,36}. However, it is important to note that the FRAP and CUPRAC responses could not be modeled within this experimental design (Table 2). Moreover, the liquid-solid ratio exerted an increased decrease effect on antioxidant activities (Figs. 1b, 1c, and 1d). Table 1 shows the relationship between temperature and the decrease in DPPH value when the β -CD concentration (1%) and the liquid-solid ratio (20) were kept constant. With regard to the extraction process with β -CD using DES solvent, an initial rise in antiradical activity up to 55°C is observed, which corresponds to an enhancement in binding affinity. This is followed by a subsequent decrease up to 80°C³⁷. The observed decline in antioxidant activities with increasing β -CD concentration likely results from the concurrent decrease in TPC extraction, indicating a limitation of higher β -CD concentrations in maximizing antioxidant recovery. Contrary to our findings, previous studies have reported significantly higher antiradical activities in samples extracted with β -CD from red beet and peach pomace compared to those extracted with ethanol^{14,18}. This discrepancy may be attributed to variations in extraction temperature, time, and method, as these parameters critically affect the efficiency of compound release, solubility, and stability during extraction.

Increases were observed in both TPC and DPPH values with the increase in the liquid-solid ratio (Fig. 3a, 3b). The process of extracting bioactive compounds with antioxidant properties, like phenolics and flavonoids, is mainly driven by the difference in concentrations between the solvent and the solid matrix³⁸. The gradient is enhanced by an increase in the liquid-to-solid ratio, which facilitates mass transfer by promoting the diffusion of the solvent into the solid matrix. Higher extraction yields, which are typically assessed through antiradical activity, are achieved due to this improved diffusion efficiency^{39,40}.

3.3.TFC

TFC values in elderberry extracts ranged from 14,497 to 20,521 mg CE/kg dm (Fig. 2a). The highest TFC was observed in Sample 2 (2% β -CD, 30% liquid-solid, 90 min) and Sample 6 (2% β -CD, 10%, 90 min), while the lowest value was found in Sample 7 (1% β -CD, 30%, 60 min) (Table 1).

RSM showed that a reduced quadratic model best explained the TFC response (Table 2). Of the model terms, only the quadratic effect of β -CD concentration (A^2) was statistically significant ($p = 0.0135$), indicating a distinct curvature and an optimal point near approximately 2% β -CD. The effects of the liquid-solid ratio and extraction time (both main and interaction effects) were not significant; therefore, the impact of time within 30–90 minutes was limited, and the influence of the liquid-solid ratio appeared secondary to β -CD concentration.

In general, reviews of flavonoid/phenolic extraction emphasise the pivotal role of the liquid-to-solid ratio and extraction time. Deviating from the optimal range can result in diminished yields due to dilution at high liquid-to-solid ratios or mass-transfer limitations at low liquid-to-solid ratios (along with thermal and degradative losses with extended duration), even within green-solvent systems^{41–43}.

The contour plot (Fig. 3c) shows that TFC approaches a maximum at ~2% β -CD and moderate liquid-solid ratios (a broad plateau roughly across 50–70%), while the response slightly declines at very low (30%) or very high (90%) ratios. Our experimental maximum (Sample 2) lies within this “~2% β -CD–low/moderate liquid-solid” region, consistent with the model’s trend. Increasing β -CD concentration up to a moderate range and employing moderate liquid-solid (L/S) ratios have been reported across multiple matrices to enhance TFC yield^{29,30,44,45}.

This pattern has also been reported in comparative β -CD-assisted extraction studies: increasing β -CD to an optimal window and employing moderate liquid-solid ratios enhance TFC yield, whereas excessive β -CD may reduce free water and limit mass transfer³⁷.

3.4.TMAC

The TMAC of elderberry extracts ranged from 7.399 to 9.701 mg C3G/kg dm (Fig. 2b). The highest TMAC was recorded for Sample 11 (3% β -CD, 10% liquid-to-solid ratio, 60 min), followed by Sample 6 (2% β -CD, 10%, 90 min). The lowest TMAC was observed in Sample 5 (1% β -CD, 20%, 30 min). In general, higher anthocyanin levels were associated with a low liquid-to-solid ratio (10%) and medium-to-high β -CD concentrations (2–3%), consistent with solid-liquid extraction behavior in which moving outside the optimal L/S window depresses yields via dilution at high L/S or mass-transfer limits at very low L/S^{46,47}.

RSM analysis (Table 2) indicated that TMAC was best described by a linear model with limited predictive power; linear and interaction terms were not statistically significant ($p > 0.05$). Unexplained variance may arise from anthocyanin-protein interactions and degradation during processing/storage⁴⁸.

The contour plot (Fig. 3d) shows the combined effect of β -CD concentration and liquid-to-solid ratio on TMAC: TMAC increases as β -CD rises from 1–3%, particularly at 10% L/S, whereas at higher L/S (20–30%) values are generally lower—again consistent with dilution and reduced β -CD-anthocyanin encounter frequency⁴⁹.

The enhancement at moderate-to-high β -CD aligns with studies showing that β -CD (or HP- β -CD) forms inclusion complexes with cyanidin-type anthocyanins, improving solubility and protecting against thermal/oxidative or gastrointestinal degradation^{12,50}. Xie et al. (2019) reported that β -cyclodextrin (β -CD) achieved higher anthocyanin extraction yields than water or aqueous ethanol across all tested mulberry cultivars; molecular docking further showed that cyanidin-3-O-glucoside is encapsulated within the β -CD cavity, thereby enhancing anthocyanin solubility.

3.5.TPAC

The TPAC values obtained in this study ranged from 17.98 to 29.72 mg C3G/kg dm (Fig. 2c). The highest TPAC was recorded in Sample 14 (3% β -CD, 10% liquid-solid ratio, 60 min), followed by Sample 2 (1% β -CD, 10%, 60 min); the lowest was observed in Sample 1 (1% β -CD, 30%, 30 min). In general, higher

values were associated with a low liquid–solid ratio (10%) and medium-to-high β -CD levels (2–3%), consistent with recent reports that stepping outside an optimal L/S window depresses yields via dilution at high L/S or mass-transfer limitations at very low L/S ^{51, 52}.

RSM (Table 2) indicated that a linear model provided the best fit for TPAC, in line with measurable but essentially linear effects of β -CD concentration and liquid–solid ratio, while curvature terms were not significant. Similar β -CD assisted extractions have identified β -CD level as a primary driver and limited curvature depending on matrix ^{18, 53}. Unexplained variance may arise from anthocyanin/proanthocyanidin–protein interactions and degradation pathways during processing or storage ^{48, 52}.

The contour plot (Fig. 3e) shows that increasing β -CD raises TPAC at low L/S (10–15%), whereas the benefit weakens above ~ 25% L/S; similar L/S-driven optima and off-optimum declines have been reported in recent RSM studies on anthocyanins ⁵⁴.

Mechanistically, proanthocyanidins belong to the less-polar fraction of phenolics and interact favorably with the hydrophobic cavity of β -cyclodextrin (β -CD). Formation of β -CD inclusion complexes increases the apparent solubility and dispersibility of PAs, facilitates mass transfer, and can mitigate degradation, thereby improving recovery ^{33, 55, 56}. Consistent with this mechanism, β -/HP- β -CD–assisted extractions from grape- and vine-derived matrices routinely deliver higher polyphenol recoveries—including flavan-3-ols—than conventional solvent systems, with additional gains when β -CD level and process variables are optimized by RSM ⁴⁵. Specifically for proanthocyanidins, β -CD has been shown to enhance solubility and extraction yield, and high recoveries have been reported from sources such as grape seeds and cocoa shells ¹³.

Table 2. Results of RSM analysis of extraction conditions

Variables	TPC		DPPH		FRAP		CUPRAC		TFC		TMAC		TPA
	Regression coefficient	P	Regression coefficient	P	Regression coefficient	P	Regression coefficient	P	Regression coefficient	P	Regression coefficient	P	Reg coe
C	-	-	-	-	-	-	-	-	-	-	-33.13	0.8844	2.33
AB	1.15	0.2532	-	-	-	-	-	-	-	-	0.44	0.1116	0.52
AC	-1.65	0.1223	-	-	-	-	-	-	-786.44	0.2331	-0.28	0.2778	-0.3
BC	-1.24	0.2207	-	-	-	-	-	-	-	-	-0.07	0.7550	-0.0
A ²	-2.31	0.0551	-9.96	0.0164	-	-	-	-	-1951.91	0.0135	0.36	0.1905	0.40
B ²	-2.36	0.0516	7.29	0.0565	-	-	-	-	-	-	-0.03	0.8890	-0.2
C ²	1.13	0.2769	6.21	0.0955	-	-	-	-	-	-	-0.29	0.2690	-0.3
Model	Quadratic		Reduced Quadratic		-		-		Reduced Quadratic		Linear		Line
Lack of fit	0.0988		0.8252		-		-		0.1811		0.4391		0.61
R ²	0.9330		0.8418		-		-		0.6370		0.2375		0.36
Adj R ²	0.8124		0.7232		-		-		0.4354		0.0295		0.19
Pred R ²	-0.0100		0.4894		-		-		-0.2299		-0.5011		-0.1

A: β -cyclodextrin concentration (%), B: liquid-solid ratio (% w/v), C: extraction time (min)

3.6.Pearson correlation coefficient analysis

The results of the correlation analysis show a clear link between phenolic compounds and antioxidant capacity at different levels (Table 3). As presented in Table 3, the determined analytes exhibited linearity, with correlation coefficient (r) values well above 0.301. A positive and strong relationship was demonstrated by TPC and DPPH radical scavenging capacity (r = 0.618, p < 0.01). DPPH also showed a significant positive correlation with CUPRAC (r = 0.301, p < 0.05). Antioxidant capacity measured by the FRAP method was found to be significantly correlated with TPAC. The r value was 0.665 and the p value was < 0.01. The same was true of the correlation with CUPRAC. Here the r value was 0.465 and the p value was < 0.01. Despite the interrelatedness and occasional correlation of TPC, antioxidant activity and reducing power ^{57, 58}, their optimisation demanded discrete conditions, signifying their independence. The differences can be attributed to the properties of polyphenols themselves ^{59, 60} and the impact of the extraction process and coexisting compounds ⁶¹. A significant positive correlation was also exhibited by CUPRAC with TFC (r = 0.325, p < 0.05). In addition, a strong and significant relationship was identified between TFC and TMA levels (r = 0.553, p < 0.01). A strong positive relationship was also identified between TPAC and CUPRAC (r = 0.467, p < 0.01). These results support the decisive role of polyphenolic compounds in the antioxidant properties of the samples, which is an important finding given the potential health benefits.

Table 3
Pearson correlation coefficient among TPC, DPPH, FRAP, CUPRAC, TFC, TMAc and TPAC assays of the elderberry extracts

	TPC	DPPH	FRAP	CUPRAC	TFC	TMAc	TPAC
TPC	1						
DPPH	0.618**	1					
FRAP	-	-	1				
CUPRAC	-	0.301*	0.465**	1			
TFC	-	-	-	0.325*	1		
TMAc	-	-	-	-	0.553**	1	
TPAC	-	-	0.665**	0.467**	-	-	1
**Correlation is significant at the 0.01 level							
*Correlation is significant at the 0.05 level							

4. Conclusions

The application of β -CD-assisted extraction in the analysis of phenolic subclasses from elderberry exhibited a high degree of efficiency, with the recovery process demonstrating a notable degree of specificity for individual compounds. TPC increased under moderate β -CD conditions, while TFC exhibited a clear optimum at approximately 2% β -CD; extraction time (30–90 min) was secondary. Optimising the concentration of β -CD and the liquid-to-solid ratio can significantly improve the extraction efficiency of phenolic compounds from elderberries. It was found that an optimal β -CD level – particularly around 2% – combined with a moderate liquid-to-solid ratio, was critical for maximising TPC, flavonoid yield and antioxidant activity. Conversely, excessive β -CD concentrations were shown to hinder mass transfer, thereby reducing extraction performance. Antioxidant responses exhibited significant correlation with assay chemistry, manifesting as stronger trends for DPPH than for FRAP/CUPRAC. The models of TMAc and TPAC demonstrated a linear relationship across the range of the experiment; therefore, their application is more suited to providing directional insight than to precise prediction. Synthesizing the model-adequacy assessment: TPC (quadratic), TFC (reduced quadratic, optimum $\sim$ 2% β -CD), and trend-based interpretations for the remaining responses converge on a practical window of $\sim$ 2% β -CD, low-to-moderate L/S ($\approx$ 10–25%), and 60–90 min to balance yields while avoiding dilution and solubility limits. Overall, these findings suggest that β -CD-assisted extraction is an effective, potentially superior alternative to conventional methods for obtaining stable, antioxidant-rich plant extracts. It is recommended that future work should take the following three forms: firstly, the validation of β -CD–phenolic inclusion and complex stability; secondly, the expansion of factor space (temperature, pH, particle size, gentle pre-treatments) and the benchmarking of alternative CDs/green co-solvents; and thirdly, the performance of multi-response optimisation with external validation alongside stability and scale-up assessments (techno-economics/LCA) to support industrial implementation.

Declarations

Conflict of interest

The authors declared no conflict of interest.

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Author Contribution

V.E. and A.A. contributed to the conceptualization, methodology, validation, formal analysis, investigation, and visualization. V.E. and A.A. also wrote and reviewed the manuscript. All authors approved the final version of the manuscript.

Data Availability

Data will be made available on request.

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Figures

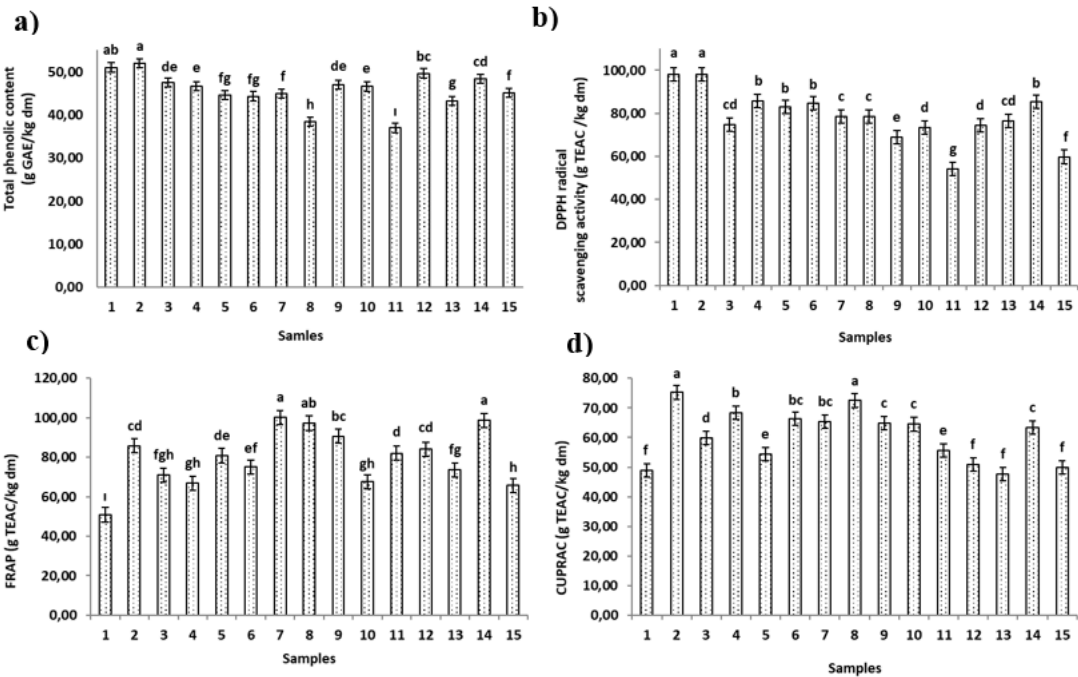


Figure 1
Results of total phenolics content and antioxidant activity assays

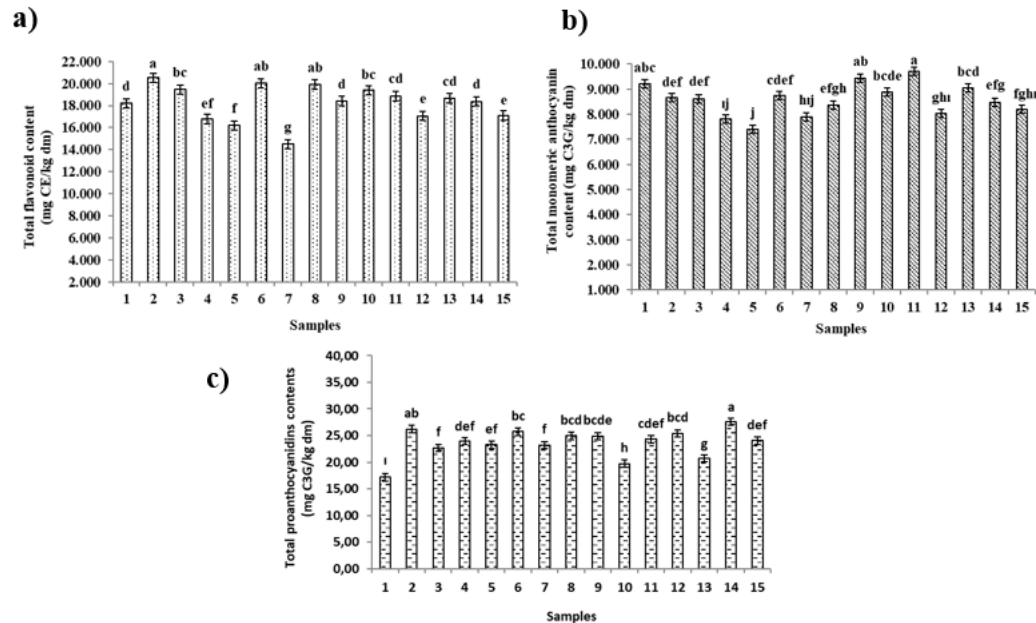


Figure 2
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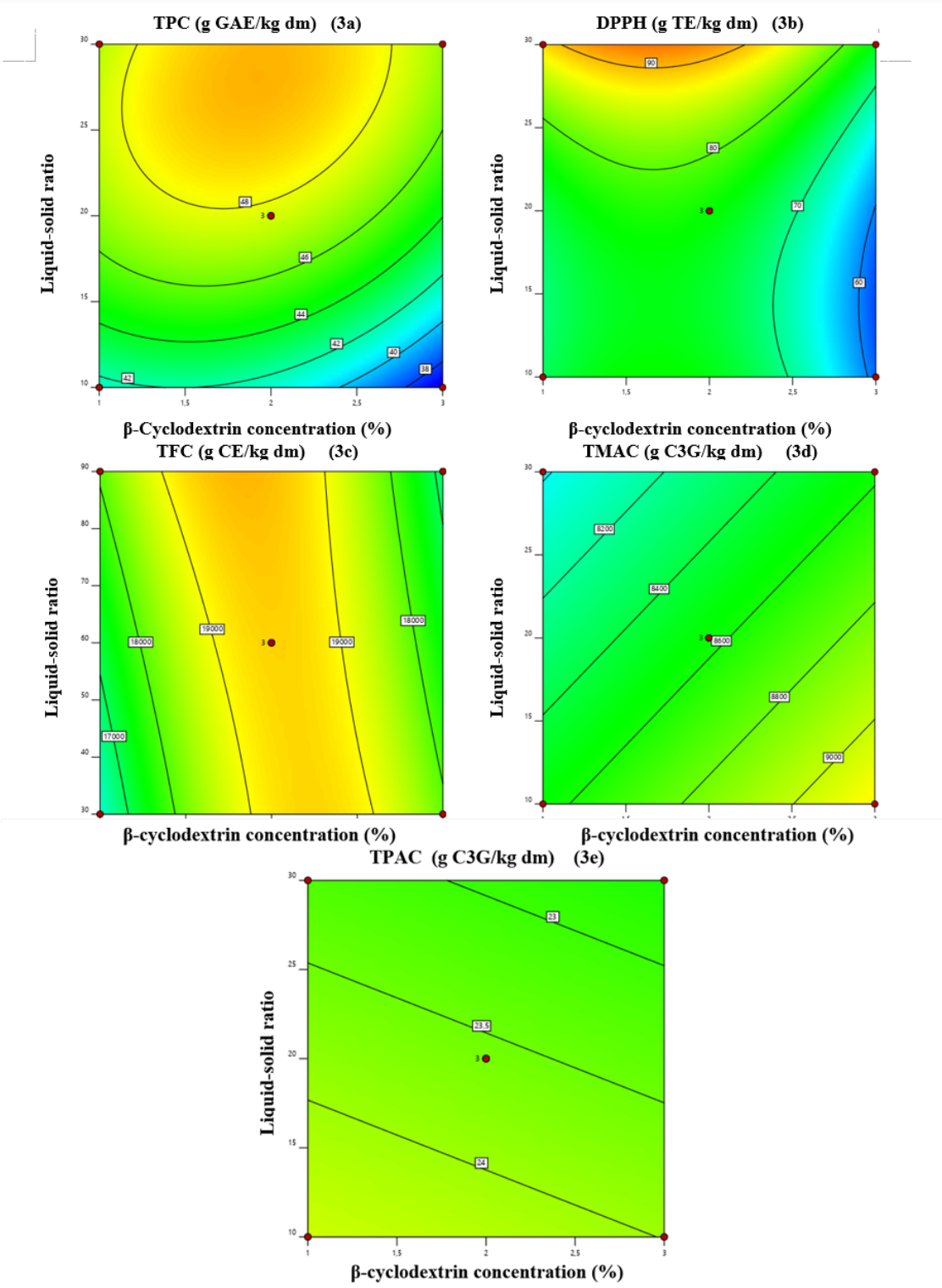


Figure 3

Results modeled by RSM analysis under specified extraction conditions