

Optimization of Extraction Parameters for Antidiabetic Bioactive Compounds from Cherry Stems (*Prunus avium*)

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Abstract

Each year, millions of tonnes of food waste are generated globally, with the majority coming from the food industry. In addition to household food waste, the recovery and sustainable use of industrial food waste is of great importance. Fruit and vegetable waste, in particular, can contain valuable compounds with various biological activities. *Prunus avium* is a widely consumed fruit with numerous industrial applications worldwide. During production processes, cherry stalks and seeds are often discarded as waste or by-products. However, cherry stems are known to exhibit various biological activities, such as diuretic, anti-diabetic, antioxidant, antifungal, anti-tumor, and hepatoprotective effects. The optimal extraction method was determined by statistical analysis of extracts obtained using solvents of different polarities, solid-liquid ratios, and extraction times. As a result of the extraction parameters optimized for antidiabetic inhibitory potential using response surface methodology, an IC_{50} value of 4.92 $\mu\text{g/ml}$ was obtained with an extraction time of 4 hours, a solid-liquid ratio of 1/30, and 40% aqueous methanol. These results demonstrate the potential of cherry stems as a dietary supplement due to their high antioxidant capacity, as well as the possibility of evaluating their bioactive molecules as active functional ingredients in functional food applications.

1. Introduction

The generation of vast amounts of waste has become an urgent global concern that requires immediate attention and intervention. Waste management in the agricultural and food industries has recently emerged as a prominent area of interest due to its significant environmental and economic implications. Agricultural waste represents a valuable source of bioactive compounds with potential applications across diverse industries, including cosmetics, nutraceuticals, and pharmaceuticals. As the demand for plant-derived bioactive compounds increases, driven by their recognised health benefits and sustainable nature, the focus has shifted towards utilizing agricultural by-products as valuable resources [1]. A well-documented illustration of this potential can be observed in the consumption of fruits and vegetables, which has been demonstrated to mitigate oxidative stress and diminish the risk of chronic diseases, including cancer and diabetes [2]. This has further stimulated the development of plant-based food products and nutritional supplements with recognised health-promoting effects [3, 4, 5].

The sweet cherry (*Prunus avium* L.) is a particularly valuable fruit due to its high concentration of bioactive compounds, with wide cultivation and consumption globally. Turkey is one of the largest producers of this fruit. In 2018, Turkey exported over 75,000 tonnes of fresh cherries, generating substantial economic returns [6]. While cherries are primarily consumed fresh or processed into products like jams and syrups, their by-products—such as pits, stems, and leaves—are often discarded, thereby contributing to environmental challenges. Among these by-products, cherry stems, also known as *Cerasorum stipites*, have been the subject of research into their medicinal properties, particularly in traditional remedies for urinary tract disorders [7, 8, 9, 10].

Cherry stems are a rich source of bioactive compounds, including hydroxycinnamic acids, flavonoids, and phenolic compounds, which have demonstrated antioxidant, antitumor, and hepatoprotective activities

[11, 12, 13, 14, 15] Traditionally used as sedatives and diuretics, cherry stems have also been employed in treating conditions such as kidney stones, edema, and hypertension [10]. Recent research has highlighted the potential antidiabetic effects of key compounds found in cherry stems, such as chlorogenic acid and quercetin. This is particularly important as diabetes, a chronic metabolic disorder characterized by elevated blood sugar levels due to insulin resistance or insufficient insulin production, continues to rise globally. Consequently, there has been growing interest in natural products with hypoglycemic properties, which tend to offer fewer side effects compared to conventional drugs [16]. Given their structural diversity and ease of availability, natural plant products are increasingly being recognized as viable alternatives to synthetic drugs [17]. In this point of view, cherry stem extracts are promising candidates for developing novel diabetes treatments [18, 19, 20]. While a considerable amount of research has been dedicated to the extraction of bioactive compounds from cherry fruits due to their antioxidant properties, the optimisation of extraction processes for cherry stems, particularly for their antidiabetic potential, remains a relatively understudied area [11].

The extraction of cherry stems frequently employs a range of techniques, including solid-liquid extraction, supercritical fluid extraction, and pressurized extraction. The majority of studies have employed ethanol or methanol as the primary solvents, with the resulting extracts exhibiting notable antioxidant activity, total phenolic content, and antimicrobial properties. However, a single solvent type has typically been employed, with the ratio of solvent to extract being a primary focus of investigation. The comparable impact of solvent type on the biological activity of cherry stem extracts has not been sufficiently explored [21, 22]. Given that solvent characteristics, such as polarity and solubility, can impact the efficiency of extraction and the profile of bioactive compounds, it is of paramount importance to optimize the choice of solvent and the specific extraction parameters to maximize the therapeutic potential of cherry stems, particularly in the context of antidiabetic applications.

Given the encouraging anti-diabetic characteristics of cherry stem compounds, there is a distinct requirement for studies to optimize the enhancement of these biological activities. While studies such as that conducted by Demir et al., have investigated factors such as ethanol concentration, temperature, and extraction time, comprehensive investigations into the effects of solvent type, solid-to-liquid ratio, and extraction time on the antidiabetic activity of cherry stem extracts remain lacking [23]. Accordingly, the principal objective of this study is to optimize the extraction conditions, with a particular focus on the solvent type, solid-to-liquid ratio and extraction time, concerning the extraction yield, antioxidant capacity, total phenol content and alpha-glucosidase inhibition. This is with a specific emphasis on the identification of compounds with potential antidiabetic effects. The objective of this research is to enhance the bioactive potential of cherry stem extracts, thereby offering insights into the creation of efficacious natural functional food for diabetes.

2. Material-Method

2.1. Material

Analytical-grade solvents (ethanol, methanol, and water) were used for the extraction process. Chemicals for antioxidant and enzyme inhibition assays, including the radical scavenging activity of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox, alpha-glucosidase enzyme, and 4-nitrophenyl-alpha-D-glucopyranoside, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cherry stems were obtained from local suppliers, dried to a moisture content below 5%, and ground to a particle size of 100–200 µm.

2.2. Design of Experiment

The Box-Behnken Design (BBD) was employed to conduct Response Surface Methodology (RSM) using Design Expert 13.0.5.0 software from Stat-Ease Inc. Three independent variables—A (Solid/Liquid Ratio), B (Solvent type), and C (Extraction time in hours) —were investigated at three levels each to optimize the extraction process, as shown in Table 1. The experimental results were evaluated in terms of various responses: extraction yield (R1), total phenol content (R2), total antioxidant capacity ABTS (R3), total antioxidant capacity DPPH (R4), and alpha-glucosidase enzyme inhibition (R5). Three factors and three central points were selected for the experimental design, resulting in a total of 15 runs conducted to evaluate the effects of these variables on the bioactivity of the extracts.

Table 1
Independent variables levels of BBD design of experiments

Factor	Parameter	Factor levels		
		-	0	+
A	Solid/Liquid Ratio	20	30	40
B	Solvent type (water, 40%methanol, ethanol)	%100 Water	%100 Ethanol	%40 Methanol
C	Time (hours)	4	14	24

Table 1 Independent variables levels of BBD design of experiments

Insert Table 1

2.3. Extraction Optimization via Response Surface Methodology (RSM)

In the extraction optimization, mixing rpm value and temperature were kept constant while other factors were implemented via the Design Expert 13 software. Three independent variables were considered: solvent type (water, ethanol, methanol), extraction time (4, 14, 24 hours), and solid-to-liquid ratio (1:20, 1:30, 1:40). The dependent responses included extraction yield, total phenolic content, antioxidant capacity (ABTS and DPPH assays), and alpha-glucosidase inhibition. A total of 15 experimental runs were conducted to evaluate the effects of these variables on the bioactivity of the extracts.

Extractions were performed in a cooled stirred incubator (NB-T205LF, N-BIOTEK). The extracts were filtered using filter paper, and the solvents were removed with a rotary evaporator (LabTech). Subsequently, the extracts were frozen at -20°C and dried in a freeze dryer (Telstar Lyoquest) at 0.1 mbar and -69°C to obtain a powdered form of extract for further analyses. After analyzing variance (ANOVA), and lack of fit, the regression coefficients for the individual linear, quadratic, and interaction terms were determined. The fit of the mathematical models was assessed by evaluating R² and adjusted R² values, confirming the model's adequacy for the study.

2.3.1. Extraction Yield

Powder extracts were used to calculate the extraction yield using Eq. 1.

$$\text{Equation 1 } \text{Extraction Yield (\%)} = \frac{\text{Total amount of extracts (g)}}{\text{Total amount of initial material (g)}} \times 100$$

2.3.2. Total Antioxidant Capacity (ABTS)

ABTS was assessed using the 96-well microplate method described by Afonso et al., 2020, with minor modifications [24]. An ABTS radical solution was created by combining 7 mM ABTS with 2.5 mM potassium persulfate in a 1:1 ratio, and the resulting mixture was kept in the dark at room temperature for 16 hours. Following this, the solution was diluted with methanol to obtain an absorbance of 0.70 at 734 nm. In each well of the microplate, 10 µL of the extract was combined with 200 µL of the freshly prepared ABTS solution and incubated in the dark at room temperature for 30 minutes [24]. The absorbance was measured at 734 nm, and the ABTS radical scavenging activity was calculated from the linear calibration curve of Trolox, provided in supplementary information (SI) Figure S1. In addition, total antioxidant capacity of extracts were expressed as IC₅₀ values.

2.3.3. Total Antioxidant Capacity (DPPH)

DPPH antioxidant activity assay was conducted via spectrophotometry, following the method described by Afonso et al., 2020 with slight adjustments [24]. The DPPH radical solution was prepared using an 80% methanol solution, and the absorbance of the DPPH radical solution was adjusted to a range of 0.9 to 1.1 at 517 nm. In a 96-well microplate, 20 µL of the extract was combined with 300 µL of the freshly prepared methanolic DPPH radical solution. The microplates were covered with foil and incubated at room temperature for 30 minutes [24]. The reduction in absorbance was then measured at 517 nm. The DPPH values of the extracts were expressed as IC₅₀ values and their total antioxidant capacity derived from the linear calibration curve of Trolox, provided in Figure S1.

2.3.4. Total Phenol Capacity

The total phenolic compounds in *P. avium* were and determined using a colorimetric assay based on procedures described by Acero et al., with some modifications. Briefly, 20 µL of the samples were added to the wells, followed by the addition of 100 µL of Folin-Ciocalteu reagent [25]. The mixture was then

incubated in the dark at room temperature for 5 minutes. After incubation, 80 μ L of a 7% sodium carbonate solution was added, and the mixture was incubated for an additional hour [25]. The absorbance was measured at 750 nm. Gallic acid was used to construct the standard curve in Figure S2.

2.3.5. Alpha-Glucosidase Enzyme Inhibition Assay

Samples were dissolved in 5% DMSO, and the alpha-glucosidase enzyme along with the 4-nitrophenyl-alpha-D-glucopyranoside substrate were prepared in a 0.1 M phosphate buffer at pH 6.9. Acarbose and the samples were added to a 96-well plate in triplicate, followed by the addition of the enzyme and incubation at 37°C for 15 minutes. Subsequently, the substrate was introduced, and the mixture was incubated for an additional 10 minutes before stopping the reaction with sodium carbonate solution. The absorbance was measured at 405 nm using a microplate reader [26]. The results are presented as IC₅₀ values (ug/ml).

3. Results

Table 2 presents a comprehensive overview of the effects of the independent variables (A, B, C) on the extraction process, inclusive of the associated experimental outcomes.

Table 2
Final version of the experimental matrix with experimental results

Factors*			Responses**					
Run number	A	B	C	R1	R2	R3	R4	R5
1	30	Water	4	48.57	43.84	127.34	108.29	29.39
2	30	Methanol	4	11.57	31.18	350.29	258	4.92
3	30	Ethanol	14	9.14	29.71	409.17	332.47	14.03
4	30	Ethanol	14	8.86	32.66	346.37	337.97	10.35
5	20	Water	14	9.57	52.10	187.64	151.75	48.85
6	40	Ethanol	24	48.43	28.45	394.95	356.20	9.56
7	40	Ethanol	4	47.29	25.55	391.9	367.93	9.98
8	30	Methanol	24	13.57	31.42	338.58	243.69	6.48
9	40	Water	14	46.43	42.64	202.67	143.78	114.9
10	40	Methanol	14	14.43	24.66	483.65	419.05	8.22
11	20	Ethanol	24	47.71	24.09	411.91	365.35	11.85
12	20	Ethanol	4	8.00	33.62	454.29	367.46	12.36
13	30	Ethanol	14	4.43	31.91	433.61	400.29	10.76
14	30	Water	24	50.00	46.87	192.33	164.58	10.64
15	20	Methanol	14	11.29	40.24	359.18	250.26	9.63
*A: Solid/Liquid Ratio B:Solvent Type C:Time (hours) ** R1: Yield %; R2 Total Antioxidant Capacity ABTS (IC50) µg/mL; R3: Total Antioxidant Capacity DPPH (µmol Trolox/g extract); R4: Total Phenol Content (mg GAE/g extract); R5: Alpha-glucosidase Inhibition IC50 (µg/mL)								

Table 2 Final version of the experimental matrix with experimental results

Insert Table 2

All key model parameters for each response were comprehensively summarized in Table 3, providing a clear and detailed overview of the variables and their respective influence on the results.

Table 3
Summary of Key Model Parameters and Their Impact on Each Response Variable

	Model	F-value	p-value	Mean	SD	Lack of fit	R ²
Extraction Yield	Reduced Quadratic	8.63	0.0085	25.29	8.38	0.0651	0.9200
Total Antioxidant Capacity (ABTS)	Reduced Cubic	54.14	0.0002	34.60	1.44	0.5938	0.9898
Total Antioxidant Capacity (DPPH)	Quadratic	7.22	0.0212	338.93	48.94	0.4616	0.9286
Total Phenol Content	Quadratic	8.18	0.0161	284.47	43.47	0.4151	0.9364
Alpha-glucosidase Inhibition	Reduced Cubic	222.57	0.0004	18.92	1.65	0.9842	0.9988

Table 3 Summary of Key Model Parameters and Their Impact on Each Response Variable

Insert Table 3

3.1. Extraction yield

A quadratic model was selected for optimizing extraction yield (%), based on the model R² and lack of fit tests. The regression coefficient (R² = 0.92) depicts that the model explains 92% of the variance in the dependent variable. The insignificance of the "lack of fit" (p > 0.05) indicates that the model provides a good fit to the data, with no significant lack of fit detected. The statistical analysis of the extraction yield of *P. avium* stems revealed that the regression model was statistically significant (p = 0.0085), which is shown in Table 3. In consideration of the p-values associated with the model terms, it can be concluded that the solid/liquid ratio (A) and solvent type (B) represent the most significant linear terms. Additionally, the quadratic term of time (C²) is also identified as a significant factor. ANOVA and fit statistics tables of the extraction yield are given in Tables S1 and S2, respectively. The results were modeled according to Eq. (2), leading to the following equation with coded factors as follows:

$$\text{Equation (2) Extraction Yield (\%)} = 7.47667 + 10.0013 * A - 12.9638 * B + 5.535 * C - 8.43 * AB - 9.6425 * AC + 9.94167 * A^2 + 3.01167 * B^2 + 20.4392 * C^2$$

Figure 1 presents a three-dimensional (3D) response surface plot, which illustrates the significant effects generated for each pair of factors. The graphs illustrate the impact of two variables on the extraction yield, while the third variable maintained constant at the center point.

Insert Fig. 1

Figure 1 Response surface model 3D plots showing the effects of A, B, and C on the extraction yield. a) Effect of C and A on extraction yield; b) Effect of A and B on extraction yield.

Figure 1A illustrates the distribution of yield values for the solid/ liquid ratio (A) and time (C) factors. It can be seen that there is a tendency for an increase in yield as the total process time increases. In particular, higher solid/liquid ratios have the potential to increase yield over a given time period. Figure 1B shows the relationship between solvent type (B) and solid/liquid ratio (A), with a particular focus on the effects of water (-1), methanol (1), and ethanol (0). It has been demonstrated that alterations in the solvent type have a considerable effect on the yield. It is noteworthy that in conditions where the solid/liquid ratio is increased, the use of water as the extraction solvent results in the highest increase in yield, followed by ethanol and finally methanol. This suggests that the characteristics of the solvent are of significant importance in determining the yield. In addition, the appropriateness of both models was assessed using several diagnostic plots. The predicted values for both responses were in appropriate agreement with the actual data given in Figure S3 and S4.

3.2. Total Antioxidant Capacity (ABTS)

A quadratic model was suggested for total antioxidant capacity (ABTS) results, however, the predicted R^2 value is not as high as we expected. To enhance the model's performance and improve its interpretability, the cubic model was chosen and certain terms were excluded from the model. Specifically, the terms (BC, A^2 and A^2B) were removed due to their lack of significance. This adjustment led to a regression coefficient $R^2 = 0.9898$ and adjusted $R^2 = 0.9716$ while maintaining a robust fit to the data. The modifications resulted in an improved predicted R^2 , thus enhancing the model's predictive capability. The reduced cubic model is statistically significant ($p < 0.05$), illustrated in Table 3. The results of the analysis of variance (ANOVA) and the fit statistic table for the total antioxidant capacity are presented in Table S3 and S4, respectively. The results were modeled in accordance with Eq. (3), resulting in the following equation with coded factors:

$$\text{Equation (3). Total antioxidant capacity (ABTS)} = 30.84 - 0.9275*A - 7.24*B + 0.8175*C - 1.53*AB + 3.11*AC + 9.52*B^2 - 2.47*C^2 - 2.47*A^2C - 5.33*AB^2$$

Figure 2 presents a three-dimensional response surface plot of total antioxidant capacity. The graphs demonstrate the influence of two variables on the extraction yield, with the third variable held at a constant value at the central point.

Insert Fig. 2

Figure 2 Response surface model 3D plots showing the effects of A, B and C on the total antioxidant capacity. a) Effect of A and B on total antioxidant capacity; b) Effect of A and C on total antioxidant capacity.

Models and three-dimensional graphs have been constructed using the IC_{50} values obtained for the total antioxidant capacity. As can be seen in Fig. 2A, the type of solvent and the solid-to-liquid ratio have a significant effect on the antioxidant capacity. The lowest IC_{50} value was identified under experimental conditions using methanol (40%) as the solvent and a solid-to-liquid ratio of 1:40. Moreover, it was found

that as the solid-to-liquid ratio increased with methanol, the IC₅₀ value decreased. In contrast, the use of water as a solvent resulted in a larger IC₅₀ value, while the use of ethanol led to an increased IC₅₀ value. The choice of solvent significantly influences the antioxidant capacity, as demonstrated by the lower IC₅₀ values observed with methanol, which effectively dissolves various phenolic and flavonoid compounds. In contrast, the higher IC₅₀ values associated with water and ethanol suggest that these solvents may be less effective in solubilizing key antioxidants, thereby limiting their reactivity and overall antioxidant activity [27]. Figure 2B illustrates the effects of time and solid-to-liquid ratio. The results demonstrate that as the solid-to-liquid ratio increases and the time decreases, the IC₅₀ value decreases which indicates a higher antioxidant capacity. In addition, the appropriateness of both models was assessed using several diagnostic plots. The predicted values for both responses were in appropriate agreement with the actual data given in Figures S5 and S6.

3.2.2. Total Antioxidant Capacity (DPPH)

A quadratic model was selected for the estimation of total antioxidant capacity via the DPPH method, based on the results of the sequential model sum of squares and lack of fit tests. The regression coefficient ($R^2 = 0.9286$) indicates that the model explains 92.86% of the variance in the dependent variable, and the insignificance of the "lack of fit" test ($p = 0.4616$) suggests that the model provides a good fit to the data, with no significant lack of fit detected. Solvent type (B) and the quadratic term of time (B^2) are the most statistically significant terms. The statistical analysis of the total antioxidant capacity via the DPPH method is presented in Table 3. Additionally, the results of the ANOVA and the fit statistics are provided in Tables S5 and S6, respectively. Figure 3 presents a 3D response surface plot of total antioxidant capacity via the DPPH method. The results were modeled according to Eq. (4), leading to the following equation with coded factors as follows:

$$\text{Equation (4). Total antioxidant capacity (DPPH)} = 396.383 + 7.51875*A + 102.715*B + 1.74375*C + 27.36*AB + 11.3575*AC - 19.175*BC + 36.5146*A^2 - 124.613*B^2 - 19.6354*C^2$$

Insert Fig. 3

Figure 3 Response surface model 3D plots showing the effects of A, B, and C on the total antioxidant capacity. a) Effect of B and C on total antioxidant capacity; b) Effect on A and B on total total antioxidant capacity and c) Effect on A and C on total total antioxidant capacity.

Figure 3A illustrates the relationship between antioxidant capacity and the B (solvent type) and C (time) variables. The lowest antioxidant capacity was observed when water was used as the extraction solvent under the shortest extraction time. When comparing antioxidant capacity based on solvent type, it is evident that ethanol and methanol have a significantly higher effect than water. The highest antioxidant capacity was achieved with methanol as the solvent, under a 1:40 solid-to-liquid ratio and 14 hours of extraction time. In Fig. 3B, as the solid-to-liquid ratio increases with water as the solvent, the antioxidant capacity decreases, reflecting lower values. In contrast, the highest antioxidant capacity was reached at a 1:40 solid-to-liquid ratio using methanol. Figure 3C depicts the relationship between variables A

(solid/liquid ratio) and C (time) with a constant B value representing ethanol. It shows that antioxidant capacity consistently remains near 400 or higher across all experimental conditions when ethanol is used as the solvent. As a result of the 3D graphs of antioxidant capacity with ABTS and DPPH method it is depicted that the most significant experimental parameter is solvent. This is because the crude extracts comprise complex mixtures of various classes of natural compounds, including phenols, which exhibit selective solubility in varying solvents. In this regard, solvent polarity exerts a pivotal influence on the extent of compounds' solubility [28]. In addition, the appropriateness of both models was assessed using several diagnostic plots. The predicted values for both responses were in appropriate agreement with the actual data given in Figure S7 and S8.

3.4. Total Phenol Content

A quadratic model was selected for the estimation of total phenol content based on the regression analysis. The regression coefficient ($R^2 = 0.9364$) indicates that the model is suitable to explain the independent variables. Furthermore, the "lack of fit" test is insignificant thus the model provides a good fit to the data. The most statistically significant terms are solvent type (B) and the quadratic term of time (B^2). The statistical analysis of the total phenol capacity is presented in Table 3. Furthermore, the results of the ANOVA and the fit statistics are presented in Table S7 and S8, respectively. Figure 4 depicts a three-dimensional response surface plot of total phenol content. The results were modeled according to Eq. (5), leading to the following equation with coded factors as follows:

$$\text{Equation (5). Total phenol content} = 356.89 + 19.0175*A + 75.325*B + 3.5175*C + 44.19*AB - 2.405*AC - 17.65*BC + 27.4475*A^2 - 143.148*B^2 - 20.11*C^2$$

Insert Fig. 4.

Figure 4. Response surface model 3D plots showing the effects of A, B and C on the total phenol content. a) Effect of B and C on total phenol capacity; b) Effect on A and B of total total phenol content and c) Effect on A and C of total total phenol content.

Figure 4A indicates that the lowest phenol content was observed when water was used as the extraction solvent with the 1/40 solid/liquid ratio. When comparing phenol content based on solvent type, ethanol, and methanol extractions were given a relatively higher phenol content than the water extraction. The highest phenol content was achieved with methanol as the solvent, under a 1:40 solid-to-liquid ratio and 14 hours of extraction time. In Fig. 4B, phenol content increases as the extraction time extends; however, at a certain point, the phenolic content tends to plateau and remain constant. Figure 4C depicts the relationship between variables A and C with a constant B value representing ethanol. It shows that phenol content changes between 300–400 (mg GAE/g extract) across all experimental conditions when ethanol is used as the solvent. In addition, the appropriateness of both models was assessed using several diagnostic plots. The predicted values for both responses were in appropriate agreement with the actual data given in Figure S9 and S10.

3.5. Alpha Glucosidase Inhibition

A quadratic model was suggested for alpha-glucosidase activity results, however the model is insignificant so to improve the model's performance and its interpretability, the cubic model was chosen and certain terms were excluded from the model. This adjustment led to a regression coefficient $R^2 = 0.9888$; adjusted $R^2 = 0.9943$ while maintaining a robust fit to the data which are given in Table 3. Furthermore, the results of the ANOVA and the fit statistics are presented in Table S9 and S10, respectively. Figure 5 depicts a three-dimensional response surface plot of alpha glucosidase inhibition. The results were modeled according to Eq. (5), leading to the following equation with coded factors as follows:

$$\text{Equation (5). Alpha glucosidase inhibition} = 11.7133 + 1.1675 * A - 7.1575 * B + 4.30 * C - 9.82 * AB + 5.0775 * BC + 12.36 * A^2 + 14.28 * B^2 - 13.14 * C^2 - 22.27 * A^2 B + 4.06 A^2 C + 17.3275 * AB^2$$

Insert Fig. 5.

Figure 5. Response surface model 3D plots showing the effects of A, B and C on the alpha-glucosidase inhibition a) Effect of A and B on alpha-glucosidase inhibition; b) Effect of B and C on alpha-glucosidase inhibition

Figure 5A shows the variation of alpha-glucosidase inhibition (IC_{50}) values depending on the A (solid/liquid ratio) and B (solvent type) factors. Notably, as the solid/liquid ratio increases in water extraction, the lowest IC_{50} value was found in methanol extraction with 1/30 solid/liquid ratio for 4 hours. Figure 5B shows the variation of alpha-glucosidase inhibition IC_{50} values depending on the B (solvent type) and C (time). This graph examines changes in IC_{50} values over time. It is observed that inhibition decreases with longer extraction times, leading to higher IC_{50} values, and particularly after a certain time, maximum inhibition levels are reached. In addition, the appropriateness of both models was assessed using several diagnostic plots. The predicted values for both responses were in appropriate agreement with the actual data given in Figure S11 and S12.

Numerical optimization was performed to enhance the inhibition potential of the alpha-glucosidase enzyme. The IC_{50} value of the extracts, which represents the concentration required to inhibit 50% of the enzyme's activity under specific conditions, serves as a key indicator of this potential; thus, a lower IC_{50} value reflects a stronger inhibitory effect on the alpha-glucosidase enzyme. In this optimization process, the solid/liquid ratio, solvent type, and extraction time were kept constant within the investigated range to achieve the minimum IC_{50} value for the alpha-glucosidase inhibition assay. The ramp graphs of the obtained results are shown in Fig. 6.

Insert Fig. 6

Figure 6 Ramps Graphs of IC_{50} Values for Alpha-Glucosidase Inhibition a) Solid/Liquid ratio b) Solvent type c) Time d) Yield e) ABTS(IC_{50}) Value f) DPPH Value g) Total Phenol Content h) Alpha-glucosidase inhibition (IC_{50}) Value

The ramps plots shown in Fig. 6 are labeled with a specific point representing the optimum value for each variable studied. The parameters used to optimize the extraction, including solid/liquid ratio, solvent type and extraction time, were kept constant within a specified range and are indicated by red points on the graph. The optimized IC_{50} value for alpha-glucosidase inhibition is marked with a blue point on the ramps plots, while other responses (yield, ABTS, DPPH, total phenolic content) were excluded from the numerical optimization and are represented by gray points. The desirability function was set to a value of 1. To obtain the highest alpha-glucosidase enzyme inhibition, i.e. the lowest IC_{50} value, optimal conditions were determined as 24.0978 solid/liquid ratio, 0.28044 solvent type (ethanol) and 23.9745 h extraction time. Under these conditions the predicted results are a yield of 34.57%, an ABTS (IC_{50}) value of 26.27, a DPPH value of 389.296 $\mu\text{mol Trolox/g}$ extract, a total phenolic content of 337.76 mg GAE/g extract and an alpha-glucosidase IC_{50} value of 0.272 $\mu\text{g/ml}$.

4. Discussion

4.1. Extraction Yield

Extraction yield is influenced by factors such as temperature, solid-to-liquid ratio, duration, solvent type, and polarity. While cherry fruits and leaves have traditionally been used for extraction, in recent years cherry stems have also been included in extraction processes due to their rich bioactive content. In this optimization study, the extraction yield of cherry stems ranged from 4.43–50%. In a study by Nunes et al. cherry stem extracts were prepared using both an aqueous infusion technique (1 g/100 mL at 100°C for 3 minutes) and a hydroethanolic extraction method (50% ethanol, 30 minutes sonication, 2 hours maceration at 25°C with stirring at 200 rpm followed by another 30 minutes sonication). The yield for the aqueous infusion of the stems was $28.42\% \pm 0.86$, while the yield for the hydroethanolic extraction was $28.89\% \pm 0.14$ [29]. In another study, *P. avium* stem extracts were prepared using an aqueous infusion technique (1 g/100 mL at 100°C for 5 min) with a yield of $26.00\% \pm 1.80\%$, while the yield for the hydroethanolic extracts was $30.42\% \pm 1.96\%$ [12]. In another study by Nastić et al. cherry stems were extracted using supercritical fluid extraction (7–15% ethanol, at 40°C, 150 and 300 bar, for 1 hour) and the yields ranged from 1.42–4.42%. In the same study, the pressurized liquid extraction method (0–100% ethanol, at 40–200°C, for 20 minutes) was investigated and the yield values varied between 10.03% and 37.31% [10]. In light of this literature, the extraction of cherry stems using different extraction processes leads to variations in yield values. Therefore, in order to improve the future use of cherry stems, it is crucial to determine their bioactivities and optimize the extraction conditions, as this plays a significant role in improving the efficacy of the final product.

4.2. Total Antioxidant Capacity and Phenol Content

The total phenol content and antioxidant properties of cherry stem extracts were evaluated using in vitro methods. The total phenol content of cherry stem extracts was measured using the Folin-Ciocalteu method, while both ABTS and DPPH assays were used to determine their antioxidant capacity. The

highest values for Total phenol content and total antioxidant capacity obtained under different experimental conditions are summarized in Table 4.

Table 4
The best extraction parameters for total phenol content, ABTS, and DPPH analysis

Bioactivity	Run number	A	B	C	Highest value
Total phenol Content (mg GAE/g extract)	10	40	Methanol	14	419.05
Antioxidant Capacity ABTS (IC ₅₀ , ug/mL)	11	20	Ethanol	24	24.09
Antioxidant Capacity DPPH (μmol Trolox/g extract)	10	40	Methanol	14	483.65

Table 4 The best extraction parameters for total phenol content, ABTS, and DPPH analysis

Insert Table 4

Table 4 presents the highest phenolic content and antioxidant capacities of cherry stem extracts. Based on these values, it can be observed that the highest antioxidant capacity via DPPH method is directly related to the high phenolic compound content. Specifically, as the phenolic content increases, the IC₅₀ value decreases, indicating stronger antioxidant activity. This relationship is further supported by the accompanying graph, which shows an inverse correlation between phenolic capacity and IC₅₀ values which is indicated in Figure S13. The presence of "-OH" groups in the structure of phenolic compounds makes them powerful antioxidants, allowing them to act as hydrogen donors and reducing agents. In this study, experiments using water as a solvent yielded lower phenolic capacity than those using ethanol and methanol. This demonstrates the importance of polarity in the extraction of phenolic compounds [30, 31].

The total phenol content, ABTS, and DPPH results have been compared with previous research, but differences in extraction systems and sample types must be taken into account for accurate interpretation. A study on sweet cherry cultivars reported total phenol content values ranging from 23.59 ± 0.14 to 32.49 ± 5.23 mg GAE/g extracts [24]. Previous studies have reported different total phenolic content values for cherry stem extract. Bursal et al. obtained a total phenol content value of 146.5 ± 13.10 mg GAE/g sample, while Ademović et al. and Babotă et al. reported total phenol content values of 121.3 ± 9.7 mg GAE/g and 37.63 ± 2.75 mg GAE/g sample, respectively [9, 3, 32]. Total phenol content of different sweet cherry cultivars was found to be 191 ± 3.10 mg GAE/g extract for Burlat, 340 ± 12.06 mg GAE/g extract for Napoleon, 244 ± 10.20 mg GAE/g extract for Coeur de pigeon and 232 ± 5.07 mg GAE/g extract for [33]. In this study, total phenol content values ranged from 108.29 to 419.05 mg GAE/g, indicating higher phenolic content due to different extraction conditions.

In terms of antioxidant capacity, Jesus et al. found IC₅₀ values of 38.29 ± 0.84 and 22.37 ± 0.29 μg/ml for infusion stem and hydroethanolic stem, respectively [12]. Similarly, in another study, the DPPH and ABTS assays for *P. avium* showed that 100% methanol yielded 96.63 ± 0.22 mg TE/g, 50% methanol yielded

82.85 ± 1.53 mg TE/g, and water yielded 252.47 ± 7.23 mg TE/g. The results of the ABTS assay showed that 100% methanol gave 116.43 ± 2.85 mg TE/g, 50% methanol gave 103.49 ± 2.11 mg TE/g and water gave 251.12 ± 5.42 mg TE/g [21]. The DPPH IC₅₀ values for different sweet cherry cultivars were 7.78 ± 0.70 µg/mL for Burlat, 2.51 ± 0.15 µg/mL for Napoleon, 5.09 ± 1.02 µg/mL for Coeur de Pigeon, 4.77 ± 0.80 µg/mL for Van and 2.95 ± 0.12 µg/mL for another cultivar. In the ABTS assay, the IC₅₀ values were 128.95 ± 16.18 µg/mL for Burlat, 55.38 ± 5.08 µg/mL for Napoleon, 126.26 ± 14.37 µg/mL for Coeur de Pigeon, 85.4 ± 8.76 µg/mL for Van and 30.86 ± 1.90 µg/mL for the other variety [33]. In another study, the DPPH IC₅₀ value was found to be 23.7 ± 0.3 µg/mL, while in our study the DPPH IC₅₀ value ranged between 25.78 and 221.82 µg/mL, as shown in Table S11 [15]. In conclusion, our total phenol content, ABTS and DPPH results are in accordance with the results reported in previous studies.

4.3. Alpha-Glucosidase Inhibition

Diabetes mellitus is a common disease resulting from abnormalities in carbohydrate metabolism associated with low blood insulin levels or insulin resistance in target organs. A key factor in carbohydrate digestion is the enzyme α-glucosidase, located at the small intestine's brush border and is responsible for breaking down carbohydrates into absorbable molecules, specifically monosaccharides [12, 29, 34]. Studies have shown that sweet cherries can inhibit α-glucosidase enzyme due to their high phenolic compounds. In one study, the potential of five different sweet cherry cultivars to inhibit α-glucosidase activity was evaluated. The IC₅₀ values for Saco, Sweetheart, Satin, Maring, and Hedelfinger were 10.79 ± 0.40, 14.34 ± 0.56, 16.31 ± 0.71, 11.38 ± 0.48 and 10.25 ± 0.49 µg/mL, respectively [35]. The α-glucosidase inhibition values for *P. avium* extracts were 25.18 ± 0.01 mmol acarbose/g for 100% methanol, 8.37 ± 0.58 mmol acarbose/g for 50% methanol and 5.10 ± 0.07 mmol acarbose/g for water [21]. In comparison with the literature, the IC₅₀ value was 3.18 ± 0.23 µg/mL for infusion extracts and 7.67 ± 0.23 µg/mL for hydroethanolic extracts [12]. The antidiabetic effect of the waste parts, such as the stalks of *P. avium*, under different extraction conditions has not been previously described. In this study, the IC₅₀ values for the α-glucosidase enzyme ranged from 4.92 to 119 µg/mL. This optimisation study showed that when water was used as the extraction solvent, the IC₅₀ value was higher, indicating lower alpha-glucosidase inhibition. This result is consistent with the literature, which reports that water-based extracts generally have a lower inhibitory potential. In conclusion, optimization of extraction methods is crucial for enhancing the bioactivity of plant extracts and may lead to more effective use of natural inhibitors in functional food applications.

5. Conclusion

This study successfully demonstrated that cherry stems can be effectively extracted using different solid/liquid ratios, extraction times, and solvent types. The results showed that the extraction process using a solid/liquid ratio of 1/30, an extraction time of 4 hours, and methanol yielded the lowest IC₅₀ value for alpha-glucosidase inhibition, making it the most effective condition. Under these optimal conditions, the extracts showed not only high phenolic content but also strong antioxidant properties. The combination of high antioxidant capacity and potential alpha-glucosidase inhibition underlines the anti-

diabetic potential of cherry stem extracts. These results suggest that cherry stem extracts can lower blood glucose levels and contain pharmacologically significant compounds. In conclusion, the results of this study indicate that cherry stem extracts should be considered not only as a natural source of antioxidants or dietary supplements but also as potential active ingredients for antidiabetic drugs. Further research is crucial to thoroughly investigate the effects of these extracts and their active components, supported by in ovo HET-CAM and in vivo experiments. Such studies will be essential to advance the development of cherry stem extracts as pharmaceutical products and may pave the way for future clinical research

Declarations

Funding

Not applicable

Ethics statement

The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and supplementary file. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Code availability

Not applicable

Author contributions

Conceptualization: OB; Methodology: OB; Formal analysis and investigation: CU, AA, and GO; Writing—original draft preparation: BTB and OB; Writing—review and editing: OB, and BTB; Funding acquisition: OB; Supervision: OB.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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Figures

Factor Coding: Actual

Yield ((%))

Design Points:

● Above Surface

○ Below Surface

4,43 50

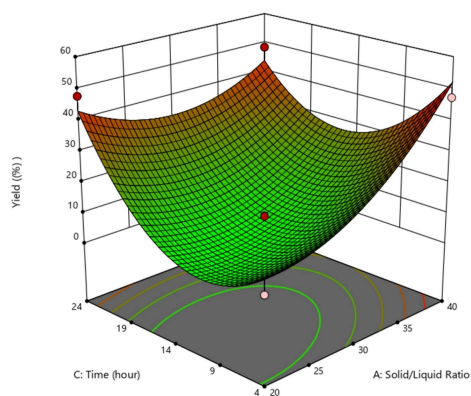
X1 = A

X2 = C

Actual Factor

B = 0

3D Surface



Factor Coding: Actual

Yield ((%))

Design Points:

● Above Surface

○ Below Surface

4,43 50

X1 = A

X2 = B

Actual Factor

C = 14

3D Surface

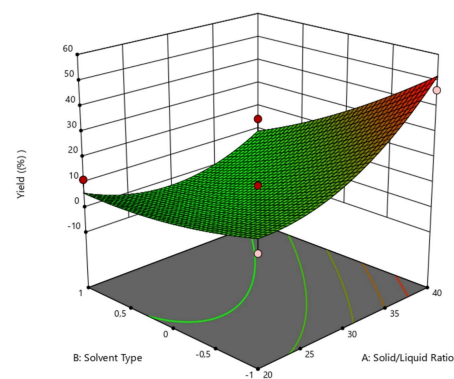


Figure 1

Response surface model 3D plots showing the effects of A, B, and C on the extraction yield. a) Effect of C and A on extraction yield; b) Effect of A and B on extraction yield.

Factor Coding: Actual

ABTS ((C50) (ug/mL))

Design Points:

● Above Surface

○ Below Surface

24,09 52,1

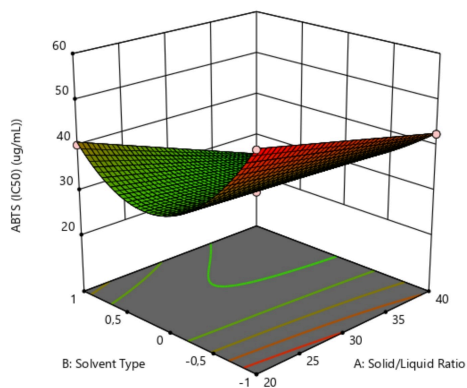
X1 = A

X2 = B

Actual Factor

C = 14

3D Surface



Factor Coding: Actual

ABTS ((C50) (ug/mL))

Design Points:

● Above Surface

○ Below Surface

24,09 52,1

X1 = A

X2 = C

Actual Factor

B = 0

3D Surface

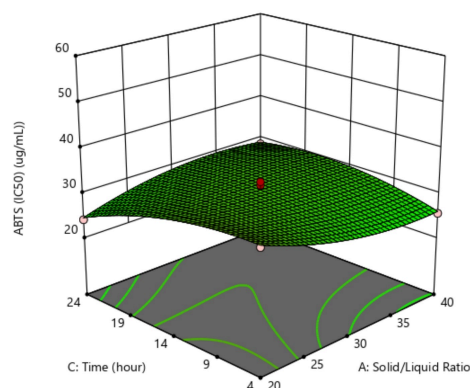


Figure 2

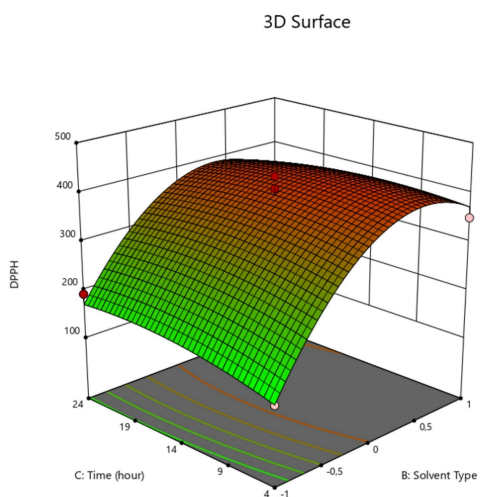
Response surface model 3D plots showing the effects of A, B and C on the total antioxidant capacity. a) Effect of A and B on total antioxidant capacity; b) Effect of A and C on total antioxidant capacity.

Factor Coding: Actual

DPPH
Design Points:
● Above Surface
○ Below Surface
127,34 483,65

X1 = B
X2 = C

Actual Factor
A = 30

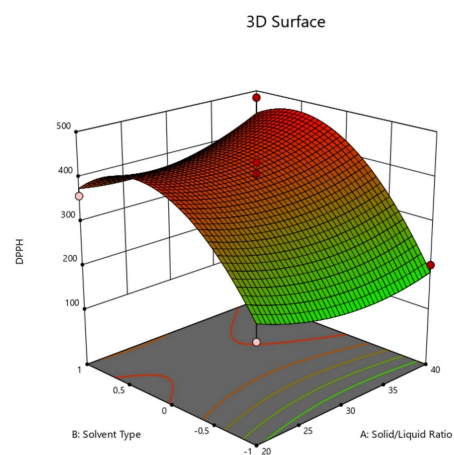


Factor Coding: Actual

DPPH
Design Points:
● Above Surface
○ Below Surface
127,34 483,65

X1 = A
X2 = B

Actual Factor
C = 14



Factor Coding: Actual

DPPH
Design Points:
● Above Surface
○ Below Surface
127,34 483,65

X1 = A
X2 = C

Actual Factor
B = 0

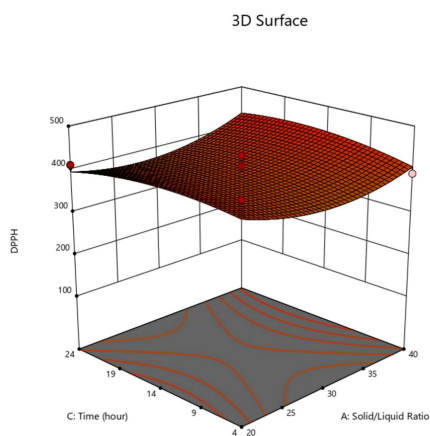


Figure 3

Response surface model 3D plots showing the effects of A, B, and C on the total antioxidant capacity. a) Effect of B and C on total antioxidant capacity; b) Effect on A and B on total total antioxidant capacity and c) Effect on A and C on total total antioxidant capacity.

Factor Coding: Actual

Total Phenol Content (mg GAE/g)

Design Points:

● Above Surface

○ Below Surface

108,29 419,05

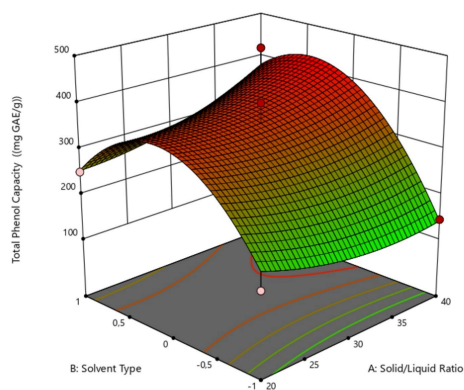
X1 = A

X2 = B

Actual Factor

C = 14

3D Surface



Factor Coding: Actual

Total Phenol Content (mg GAE/g)

Design Points:

● Above Surface

○ Below Surface

108,29 419,05

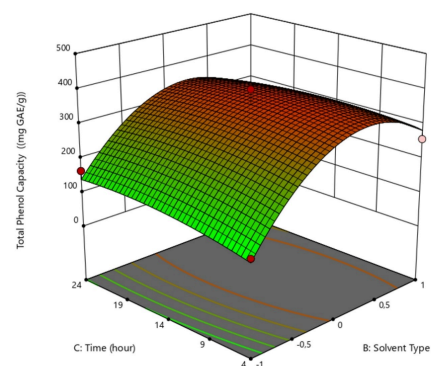
X1 = B

X2 = C

Actual Factor

A = 30

3D Surface



Factor Coding: Actual

Total Phenol Content (mg GAE/g)

Design Points:

● Above Surface

○ Below Surface

108,29 419,05

X1 = A

X2 = C

Actual Factor

B = 0

3D Surface

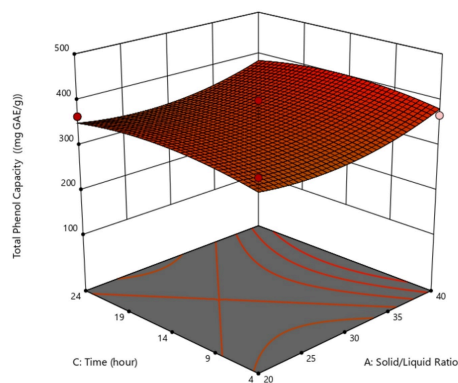


Figure 4

Response surface model 3D plots showing the effects of A, B and C on the total phenol content. a) Effect of B and C on total phenol capacity; b) Effect on A and B of total total phenol content and c) Effect on A and C of total total phenol content.

a

Factor Coding: Actual

alpha-glucosidase inhibitor (IC50) (ug/mL)

Design Points:

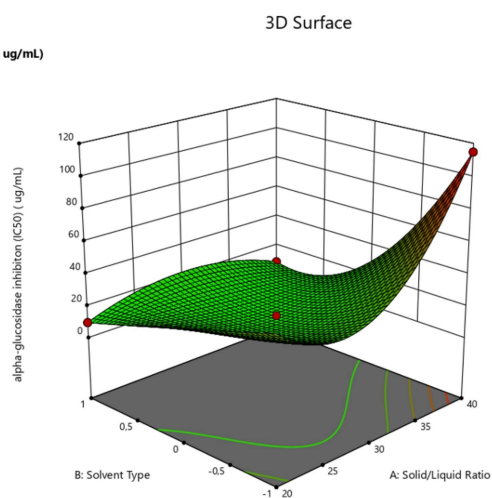
● Above Surface
○ Below Surface
4,92 114,9

X1 = A

X2 = B

Actual Factor

C = 14



b

Factor Coding: Actual

alpha-glucosidase inhibitor (IC50) (ug/mL)

Design Points:

● Above Surface
○ Below Surface
4,92 114,9

X1 = B

X2 = C

Actual Factor

A = 30

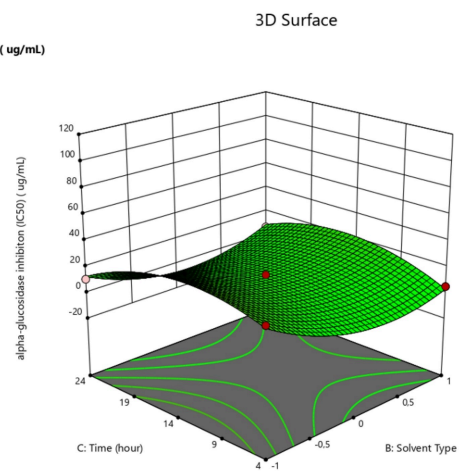


Figure 5

Response surface model 3D plots showing the effects of A, B and C on the alpha-glucosidase inhibition a) Effect of A and B on alpha-glucosidase inhibition; b) Effect of B and C on alpha-glucosidase inhibition

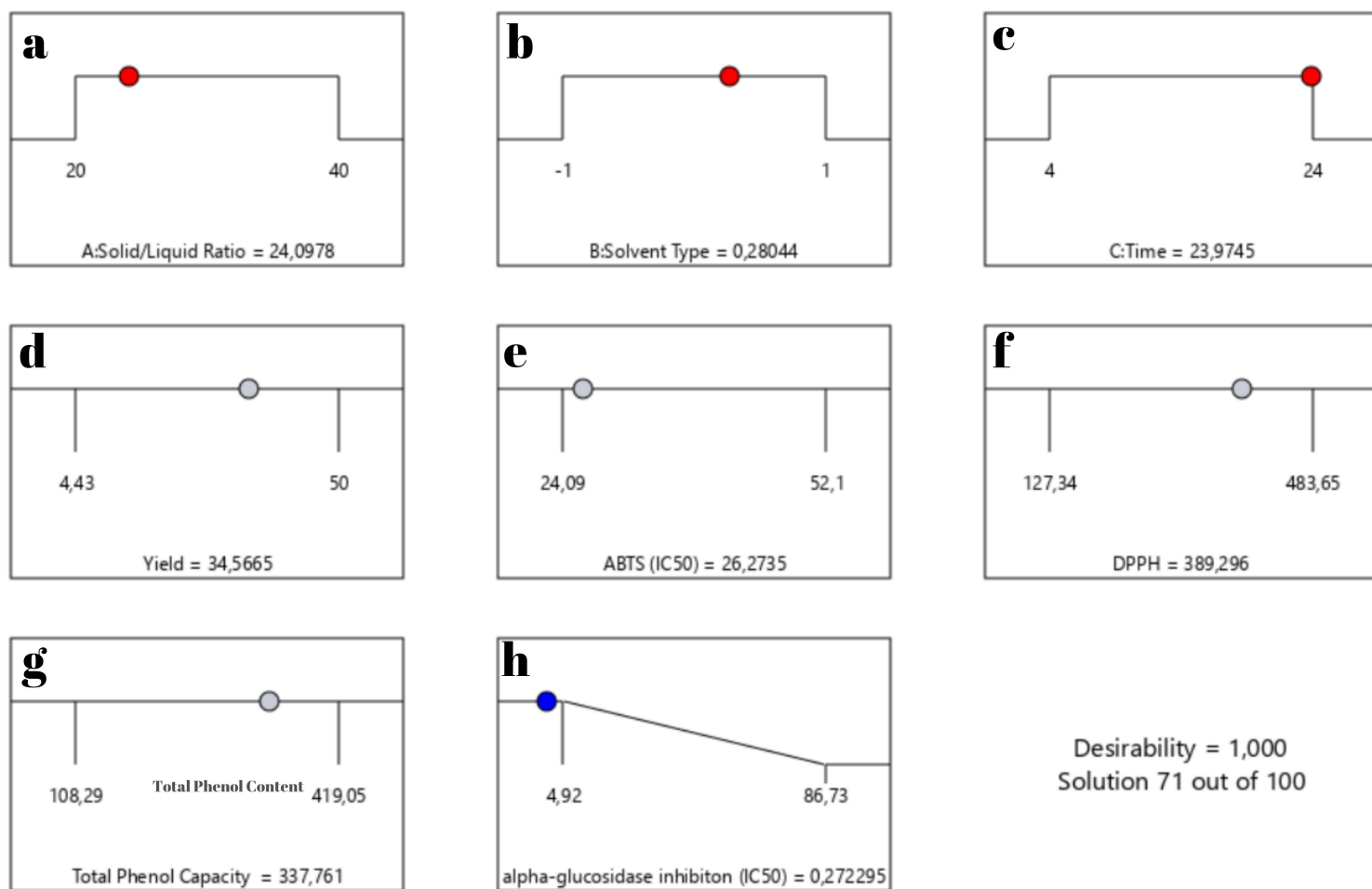


Figure 6

Ramps Graphs of IC_{50} Values for Alpha-Glucosidase Inhibition a) Solid/Liquid ratio b) Solvent type c) Time d) Yield e) ABTS(IC_{50}) Value f) DPPH Value g) Total Phenol Content h) Alpha-glucosidase inhibition (IC_{50}) Value

Supplementary Files

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- [GraphicalAbstract.jpg](#)