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# Optimization of iceplant (*Mesembryanthemum crystallinum*) polyphenols extraction conditions by Response Surface Methodology (RSM)

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***Abstract***

The influence of temperature (40 - 50°C), extraction duration (60-180 minutes), and liquid (ethanol 70%) to solid ratio (10-25 ml/g, solvent volume per g of raw material) on extractability of total phenolics and antioxidant activity of iceplant were examined. Response surface method was used to identify the best extraction conditions for the phenolics and antioxidant activity. The Central Composite Design revealed that polynomials predictive models were in good agreement with the experimental data, with correlation coefficients of 0.9476, 0.8783, 0.9646, and 0.8711 for total phenolics, DPPH, ABTS, and reducing power (RP), respectively. The optimum conditions were a ratio of 15.469 mL/g liquid (ethanol 70%) to solid, an extraction period of 120 minutes, and a temperature of 40 °C. Under optimised conditions, high levels of total phenolic compounds (9.45 mg GAE g -1) were produced, as well as high levels of DPPH (88.17%) and ABTS (57.15%) free radical scavenging ability and reducing power (15.1 mg ascorbic acid/g extract). The experimental results coincided with those predicted, showing that the utilised model was appropriate and that the response surface method was successful in optimising the extraction parameters of the examined system. This is the first publication on optimising the extraction method of phenol components and the antioxidant activity of iceplant.

***Keywords***: iceplant, RSM, *Mesembryanthemum crystallinum*, polyphenols and antioxidant activity

## 1.Introduction

Natural plants have drawn a lot of interest as sources of physiologically active compounds such antioxidants, anticarcinogens, and antimutagens [1,2]. On the other hand, there is still a dearth of scientific data on the antioxidant qualities of many plants, particularly those that are less often utilised in food and medicine. Therefore, evaluating these qualities is still fascinating and valuable, especially when looking for novel sources of nutraceuticals, functional foods, and natural antioxidants [3]. One of the most significant wild halophytes is the iceplant *(Mesembryanthemum crystallinum)*, which is found on the northern coast of Egypt [4]. It is a member of the Aizoaceae family, one of the plant groups that has gone the furthest into Africa. The plant's surface is coated in trichomes known as epiderm bladder cells, and such cells are represented in the names given to the plant, such as icicle plant or crystalline iceplant [5]. The leaves and stems of the iceplant have a juicy look and grow in thick stems that stretch out horizontally on the earth [6]. At the earliest stages of its growth, it is yellow or green, and as it ages, it turns orange. Its blossoms resemble sea anemones and range in colour from yellow to purple. When it reached maturity, it produced fig-like fruits that were 3–4 cm in diameter [7].

*Mesembryanthemum* is a functional food and medicinal herb that contains a variety of biological and health-promoting elements. It is well-known for having a high concentration of bioactive substances including polyphenols and flavonoids [8]. Additionally, it is well recognised for having antioxidant enzymes including ascorbate peroxidase, superoxide dismutase, and catalase [9]. Iceplant has been shown to have a high concentration of D-pinitol, which has been

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demonstrated to have anticancer, antiinflammatory, antioxidant and antidiabetic properties [10]. Phenolics have a variety of biochemical functions, including antimutagenic, the capacity to change gene expression, anticarcinogenic, and antioxidant capabilities [11]. The quality and amount of antioxidant activity in iceplant may be influenced by the extraction conditions, however this has never been established. The liquid-solid extraction process may be greatly impacted by a number of variables, including the solvent composition, the extraction duration, temperature, pH, solvent-solid ratio, and particle size [12]. In order to use phytochemicals in the creation of nutritional supplements or nutrients, functional food components, and additions to food, pharmaceutical, and cosmetic goods, it is now crucial to extract and purify bioactive molecules from natural sources [13]. However, the choice of the extraction factors, including temperature, stirring rate, extraction duration, specimen size of the particle, pH, and solvent/solid ratio, can significantly affect the recovery of phenols from agricultural by-products of crops [14]. There is a "one variable at a time" method for choosing the best extraction parameters, but it is quite time-consuming and does not take into account any potential interactions between variables and factors [15]. The Response Surface Methodology, also known as RSM, is a powerful statistical approach for optimising complicated systems. When compared to other designs, the Central Composite Design kind of RSM is more effective, easier to set up and comprehend the optimisation trials, and has been used extensively to optimise a large number of variables [16]. Currently, RSM is used to extract phenolic substances from a variety of plant sources [17-20]. Extraction of phenolic components is one example of a biotechnological and biochemical operation that has been effectively modelled and optimised using response surface technique. However, *Mesembryanthemum crystallinum*, sometimes known as iceplant, has received little research attention in Egypt. The current study's goal was to discover how to maximise the extraction of phenols and achieve the best in vitro antioxidant activity by adjusting extraction parameters such temperature, duration, and liquid/solid ratio.

## 2. Materials and methods

### *2.1. Materials:*

*Mesembryanthemum crystallinum*, also known as iceplant, was gathered in March 2021 from both sides of the international coastline road close to El-Boruls, in the Kafr ElSheikh Governorate of Egypt (latitude 32 35'N and longitude 31 16'E). All of the chemicals and solvents used in this investigation (HPLC grade) were purchased from Sigma Company of Chemicals and Drugs, St. Louis, MO, USA.

### *2.2. Methods:*

#### 2.2.1. Sample preparation

According to Ibtissem, *et al.* [21], iceplant powder was made as follows: iceplant samples had been rinsed with distilled water, any extra water was blotted out with a white towel, they were then left out in the open for seven days while it was dark, and finally they were dried in an oven for two hours at 60±2°C.

#### 2.2.2. Extraction of iceplant powder

The ethanolic extract from iceplant powder has been produced in accordance with Gaur, *et al.* [22] five grammes of fine Iceplant powder was combined with 70% ethanol at temperatures ranging from 30 to 50 °C ($X_1$), periods ranging from 60 to 180 minutes ($X_2$), and liquid/solid (v/w) ratios ($X_3$) ranging from 10:1 to 25:1 (Table 1). These values were chosen based on preliminary investigations. Extracts were filtered, then kept in the flask at 4°C until analysis to avoid oxidative damage. Total phenolic Content (TPC), DPPH radical scavenging capacity assay (DPPH), RP, and ABTS radical cation inhibition antioxidant assay (ABTS) were all assessed as variables of dependence (responses).

***Table 1.*** Uncoded and coded experimental independent factors of the RSM design

| Factor | Name | Units | Type | SubType | Minimum | Maximum | Coded Low | Coded High | Mean | Std. Dev. |
|---|---|---|---|---|---|---|---|---|---|---|
| A | Temperature | °C | Numeric | Continuous | 23.18 | 56.82 | -1 ↔ 30.00 | +1 ↔ 50.00 | 40.00 | 8.48 |
| B | Time | Minute | Numeric | Continuous | 19.09 | 220.91 | -1 ↔ 60.00 | +1 ↔ 180.00 | 120.00 | 50.87 |
| C | ratios liquid/solid | mL/g | Numeric | Continuous | 4.89 | 30.11 | -1 ↔ 10.00 | +1 ↔ 25.00 | 17.50 | 6.36 |

### *2.3. Experimental Design*

Response Surface Methodology (RSM) was used to optimise the experiment for the extraction of TPC and in vitro antioxidant activity (DPPH, ABTS, and RP). Twenty experimental runs totalled in the Central Composite Design (CCD), comprising six at central points, six at axial points, and eight at factorial points (Table 2).

Table 1 lists the extraction variables, which included extraction temperature ($X_1$, $^oC$), extraction duration ($X_2$, min), and liquid to solid ratio ($X_3$, mL/g, v/w). The experimental data were fitted to a second-order polynomial model to give regression coefficients ($\beta_0$). The following describes the generalised second-order polynomial model used in the response surface analysis:

$$Y = \beta_0 + \Sigma^k{}_{i=1}\, \beta_i X_i + \Sigma^k{}_{i=1}\, \beta_{ii} X_i^2 + \Sigma^k{}_{i<j=1}\, \beta_{ij} X_i X_j,$$

where Y stands for the response variable, $X_i$ and $X_j$ are the independent variables, and n is the total number of variables evaluated. The terms "$\beta_0$," "$\beta_i$," "$\beta_{ii}$," and "$\beta i_j$," respectively, denote the coefficients of constant, linear, quadratic, and interaction effects.

An analysis of variance (ANOVA) was conducted to determine the impact of each element (temperature, time, and ratio) in order to assess how well the anticipated model performed on the response variable. The regression model's fitness was further assessed using the regression coefficient ($R^2$), the regression model's p-value, and the p-value of the lack of fit (LOF). Response surfaces (3D plots) were taken into consideration while selecting the ideal circumstances. Based on the results from RSM, the optimised conditions were verified for the highest TPC and antioxidant activities (DPPH, ABTS, and RP). The optimal extraction conditions were used to determine all the responds. To assess the model's applicability, the experimental results were contrasted with the projected values based on CV%.

### *2.4. TP determination:*

The Folin-Ciocalteu technique was used to estimate the TP in the extracts, which was calculated as weight (mg) of galic acid equivalents (GAE) /g of dry iceplant powder [23]. Every single sample was tested three times.

### *2.5. In Vitro antioxidant determination:*

#### 2.5.1. Determination of DPPH Radical Scavenging Activity:

The technique outlined by Elsebaie, *et al.* [24] was used to assess the antioxidant activity of the extracts based on the scavenging activity of the $DPPH^{\cdot}$. The following formula was used to get the inhibition percentage, which represents the capacity to scavenge the $DPPH^{\cdot}$:

$$RSA\ (\%) = \frac{\text{Control absorbance} - \text{Extract absorbance}}{\text{Control absorbance}} \times 100$$

#### 2.5.2. RP determination:

The procedure outlined by Broncano, *et al.* [25] was used to determine the RP in triplicate. At 700 nm, absorbance was measured. A positive control was utilised, which was ascorbic acid (vitamin C).

The range of the vitamin C calibration curve, which was used to determine the reducing power, was 0 to 1 g/mL. Results were given in mg of ascorbic acid per gramme of extract.

#### 2.5.3. ABTS determination:

The technique described by Wołosiak, *et al.* [26], with minor changes, was used to assess the scavenging capacity against $ABTS^+$ in triplicate.

The reaction between ABTS and potassium persulfate produced the $ABTS^+$. The ABTS stock solution was created by reacting 7.4 mM of ABTS with 2.45 mM potassium persulfate (final concentration) and letting the mixture sit at room temperature for 16 hours in the dark.

The $ABTS^+$ stock solution was diluted with distilled water to produce the $ABTS^+$ operating solution, which had an absorbance of 0.7 at 734 nm. Then, 3 mL of the $ABTS^+$ operating solution was combined with 0.4 mL of the sample extract. At 734 nm, the absorbance was assessed following a 30-minute incubation. All samples' radical scavenging assay results were reported as a proportion of scavenging ABTS radicals with the following equation:

$$ABTS\ scavenging\ \text{effect}\ (\%) = \frac{\text{Control absorbance} - \text{Extract absorbance}}{\text{Control absorbance}} \times 100$$

### *2.6. Validation of the Model:*

The maximal phenolic content and in vitro antioxidant activities (DPPH, ABTS, and RP) were confirmed using RSM results, and the optimal extraction parameters (time, temperature, and liquid/solid ratio) were then determined.

All of the replies were once more calculated using the best extraction circumstances. In order to evaluate the model's validity, the experimental results were contrasted with those that the model predicted.

## 3.Results and discussion:

The results of the experiments for TPC, DPPH, ABTS, and reducing power are shown in Tables 2 and 3 along with the regression coefficients that were calculated after fitting the data from the experiments to the second order polynomial response equations.

Twenty condensed experimental sets with six replications at the independent variable's midpoints make up the experimental design (Table 2). Analysis of variance (ANOVA) was used to determine the significance of the influence of the linear, quadratic, or interaction coefficients on the given responses.

The least squares approach was used to determine the regression coefficients of the model's intercept, linear, quadratic, and interaction variables. Each component's p-value indicates the level of significance for that factor. The fitted model accurately represents the experimental data, with $R^2$ values ranging from 0.8711 to 0.9646 (Table 3).

### *3.1. Fitting the Model*

By using second order polynomial equations, the extraction procedure was optimised. With R2 values of 0.9476 for TPC, 0.8783 for DPPH, 0.9646 for ABTS, and 0.8711 for RP, respectively, the model exhibits high significance and a strong match with the experimental data (Table 3).

Table 3 shows the results of numerous linear regressions used to determine the regression coefficients for dependent variables. In addition to time ($X_2$) for DPPH and ratio v/w for all variables, temperature ($X_1$) had a negative linear influence that was significant for both TPC and RP variables.

TPC and DPPH variables were found to be significantly affected by the interaction of $X_{1-2}$ (temperature and time), all variables except ABTS were significantly affected by the interaction of $X_{1-3}$ (Temperature and ratios liquid/solid), and only the DPPH variable was significantly affected by the interaction of $X_{2-3}$ (time and ratios liquid/solid). Temperature had a strong negative quadratic influence on the ABTS and RP variables. TPC and DPPH were significantly impacted negatively by the quadratic effect of time ($X_2$). All variables, with the exception of DPPH, were shown to be significantly affected by the quadratic impact of $X_3$ (ratio v/w). The three model parameters may explain the experimental variance for response factors, as evidenced by the substantial F-value for the model and the ANOVA findings for every response factor (Table 2). The LOF was determined to be non-significant, suggesting that the model could successfully fit the experimental results for all of the variable responses (Table 3).

**Table 2.** Experimental matrix and values of observed response

| Run | A:Temperature (°C) | B:Time (minute) | C:ratios liquid/solid (mL/g) | Total phenol content (mg gallic acid/g) | DPPH (%) | ABTS (%) | Reducing Power (mg ascorbic acid/g) |
|---|---|---|---|---|---|---|---|
| **1** | 40 | 120 | 30.1134 | 5.91 | 83.4 | 36.87 | 14.98 |
| **2** | 50 | 60 | 10 | 6.01 | 76.26 | 81.15 | 13.81 |
| **3** | 30 | 180 | 10 | 7.21 | 91.2 | 72.46 | 16.12 |
| **4** | 40 | 120 | 17.5 | 5.40 | 87.08 | 49.22 | 14.22 |
| **5** | 30 | 60 | 25 | 5.38 | 38.53 | 36.82 | 14.19 |
| **6** | 40 | 19.0924 | 17.5 | 4.11 | 87.19 | 47.12 | 14.29 |
| **7** | 40 | 120 | 17.5 | 5.37 | 86.31 | 50.99 | 14.18 |
| **8** | 40 | 120 | 17.5 | 5.42 | 87.37 | 50.29 | 14.37 |
| **9** | 40 | 220.908 | 17.5 | 5.01 | 74.8 | 49.32 | 13.97 |
| **10** | 23.1821 | 120 | 17.5 | 6.81 | 94.21 | 53.13 | 15.82 |
| **11** | 40 | 120 | 17.5 | 5.40 | 86.72 | 42.29 | 14.35 |
| **12** | 40 | 120 | 17.5 | 5.38 | 86.62 | 34.02 | 14.26 |
| **13** | 50 | 60 | 25 | 4.63 | 81.48 | 36.33 | 14.43 |
| **14** | 56.8179 | 120 | 17.5 | 4.63 | 88.19 | 58.72 | 14.35 |
| **15** | 50 | 180 | 10 | 6.61 | 96.93 | 84 | 15.68 |
| **16** | 30 | 180 | 25 | 3.91 | 44.27 | 38.53 | 14.24 |
| **17** | 40 | 120 | 4.88655 | 8.51 | 92.47 | 97.91 | 15.72 |
| **18** | 40 | 120 | 17.5 | 5.43 | 88.45 | 45.94 | 14.41 |
| **19** | 30 | 60 | 10 | 8.11 | 94.48 | 76.54 | 15.90 |
| **20** | 50 | 180 | 25 | 5.38 | 83.19 | 39.61 | 14.28 |

**Table 3.** Regression coefficient (β), coefficient of determination ($R^2$), and F-test values of the predicted second order polynomial models for TPC, DPPH, ABTS and RP.

| **Regression coefficient (β)** | | | | |
|---|---|---|---|---|
| | TPC | DPPH | ABTS | RP |
| **Intercept** | | | | |
| **$X_0$** | 5.39842 | 87.1101 | 45.4489 | 14.3002 |
| **Liner** | | | | |
| **A-Temperature ($X_1$)** | **-0.413441*** | 1.06714 | 1.91415 | **-0.345778*** |
| **B-Time ($X_2$)** | 0.036144 | **-2.515737*** | 0.546242 | 0.106308 |
| **C-ratios liquid/solid ($X_3$)** | **-0.95283*** | **-6.12541*** | **-19.442*** | **-0.411114*** |
| **Cross product** | | | | |
| **AB** | **0.465*** | **7.865*** | 1.0625 | 0.18125 |
| **AC** | **0.4275*** | **6.42*** | -1.945 | **0.35125*** |
| **BC** | -0.0525 | **-6.6175*** | 0.7775 | -0.27375 |
| **Quadratic** | | | | |
| **$A^2$** | 0.12346 | -0.258675 | **3.76188*** | **0.265991*** |
| **$B^2$** | **-0.286662*** | **-5.63445*** | 1.03775 | -0.0716524 |
| **$C^2$** | **0.650254*** | 0.177964 | **7.81537*** | **0.359683*** |
| **$R^2$** | 0.9476 | 0.8783 | 0.9646 | 0.8711 |
| **F value (model)** | 20.09* | 8.02* | 30.30* | 7.51* |
| **F value (lack of fit)** | 1.47 | 3.06 | 0.0993 | 30.16 |

### *3.2. Effect of the Extraction Variables on TPC:*

With regard to the experimental data, the model displayed a high level of significance ($p<0.05$). The results of the analysis of variance (ANOVA) revealed a negative linear ($p < 0.05$) ($X_1$ and $X_3$) and quadratic ($p<0.05$) influence on TPC content (Table 3). While the interaction between $X_{1-2}$ and $X_{1-3}$ was shown to be significant on TPC, the interaction between $X_2$ and $X_3$ was not significant on the experimental results. The non-significant value of LOF ($F = 1.47$; $p<0.05$) demonstrated that the model is fit to the geographic effect of the factors on this response with decent prediction ($R^2 = 0.9476$) but is not significant when compared to the pure error.

TPC results matched those reported by Calvo, *et al.* [27], who also used ethanol as a solvent. The findings of the regression analysis (Table 3) showed that TPC extraction was, to some extent, higher at lower temperatures and lower liquid/solid ratios. Additionally, it was discovered that temperature and the solid-to-liquid ratio were the factors that were most important for the extraction of phenolic compounds from black rice [28] and grape by-products [29] and Figure 1A, B, and C show the connection between TPC and process factors. TPC was most effectively extracted between 30 and 40 °C and 10 to 13 mL/g. This conclusion is consistent with earlier research on *Schinus molle L.* peel [16] and shows that extraction time had no discernible influence on the extraction of TPC. As a result, it may be stated that the minimum extraction period (60 min) is sufficient for the current experiment's TPC extraction. As previously stated, is known to have an essential impact in polyphenols extraction effectiveness [30]. When the temperature is greater, the solubility and diffusion coefficients of the compounds increase, the viscosity and surface tension of the solvent decrease, and the phenolic-protein and phenolic-polysaccharide connections become weaker. All of the above actions would facilitate the phenolic compounds' migration into the extraction solvent [31]. On the other hand, it has been discovered that phenolic compounds are denatured by chemical or enzymatic processes above a specific temperature [29]. According to some writers, high temperatures may encourage membrane denaturation, which might affect the mobility of the solvent and solutes as well as the extraction process [32]. Under these circumstances, undesirable molecules could also dissolve [30].

According to Cacace and Mazza [33], polyphenols extraction yield decreased as temperature rose from 40 to 74 °C. According to the substantial linear negative coefficient in Table 3 for the liquid/solid (v/w) ratio, the greater this ratio, the lower the extracted TPC was. According to Liyana-Pathirana and Shahidi [34], there was also a significant quadratic impact of the liquid/solid ratio on TPC (β= -0.95283; $p<0.05$). This result indicates that the ratio had both positive and negative effects on TPC.

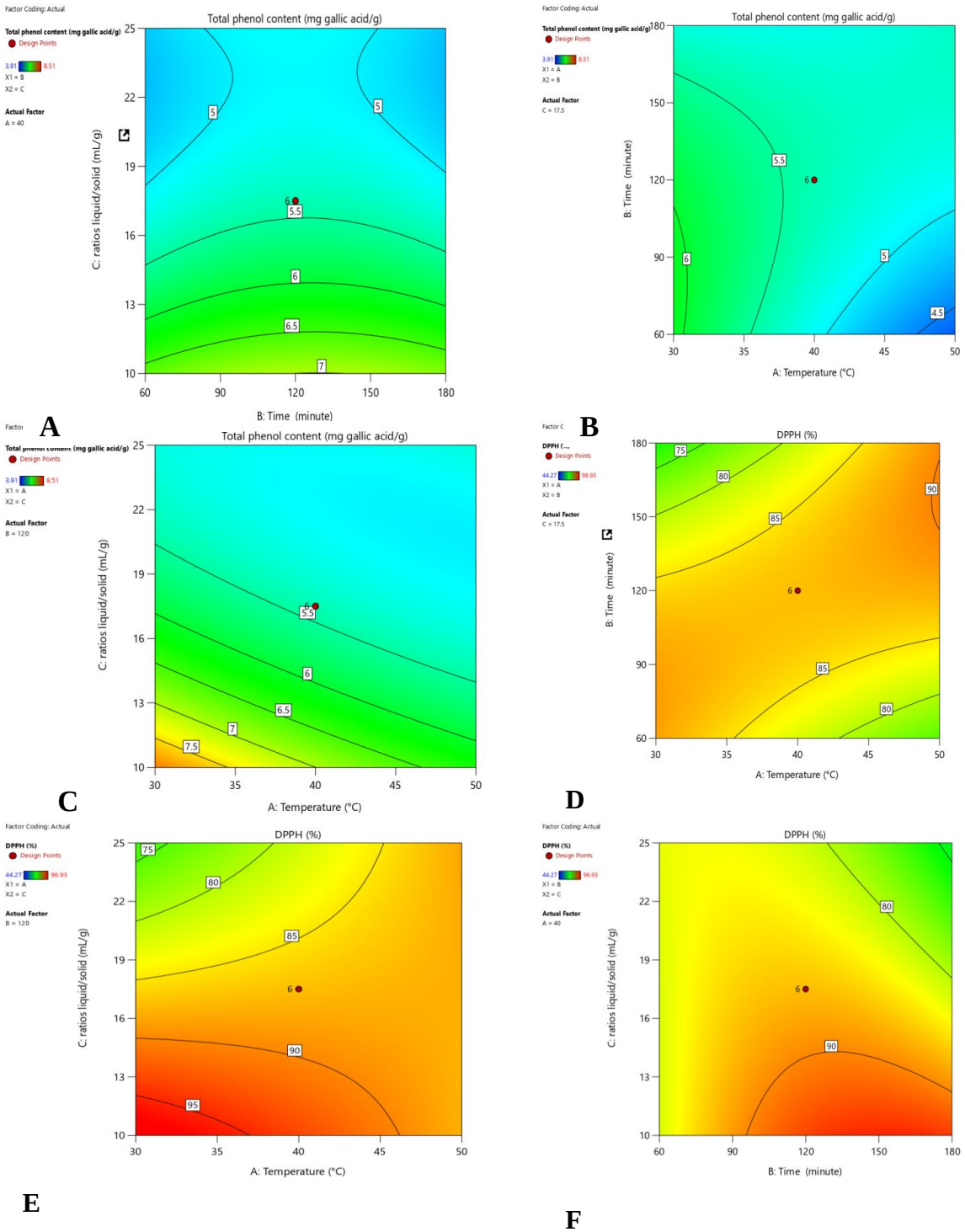


**Figure 1.** Contour plots for the effect of extraction variables on (A, B and C) total phenol content (mg gallic acid/g) and (D, E and F) DPPH %.

*3.3. Effect of the extraction variables on the antioxidant potential of iceplant extracts:*

Analysis of variance (ANOVA) results indicated that time ($X_2$), ratio ($X_3$), and time ($X_2^2$) all had significant negative linear impacts on DPPH levels ($p<0.05$) as well as negative quadratic effects. In the model, all of the variable interactions were also statistically significant ($p< 0.05$). Figures 1D, E, and F provide a visual representation of this. It can be seen that DPPH peaked at a certain time, temperature, and ratio, and then started to fall. According to Figures 1D and E and F, the maximum activity was seen between the temperatures of 30 and 40°C and between the times of 60 and 90 minutes and 10 to 13 mL/g.

Only the quadratic influence of temperature ($X_1^2$) and the linear effect of ratio liquid/solid (X3) were significant for the model in the ABTS data ($p <0.05$) (Table 3). Figure 2 B and C illustrates how ABTS values grew as the ratio shrank. These parameters have a positive correlation ($R = 0.795$; $p <0.05$), which explains this behaviour in a manner similar to that which was described for TPC (Table 4). The results published by Zhang, *et al.* [35] are in keeping with Table 4's finding that there was no significant correlation between ABTS and DPPH. It is widely known that the responses of DPPH and ABTS tests to antioxidants differ in a number of ways. As a result, ABTS may be dissolved in both aqueous and organic solvents, and because the chemicals in samples are hydrophilic and lipophilic, respectively, the antioxidant activity can be assessed in these solvents [36]. Contrarily, DPPH cannot be dissolved in aqueous solutions and must instead be done so in organic ones (particularly alcoholic ones), which poses a significant obstacle to understanding the function of hydrophilic antioxidants [36].

Table 4 displays the determined Pearson's correlation coefficient for the response variables. This table indicates that there is no discernible relationship between DPPH readings and TPC. The Folin-Ciocalteau technique may be used to detect substances other than phenols, and final absorbance may also be the consequence of reduction processes involving ascorbates and thiols [37]. However, a plant extract's antioxidant activity cannot be solely correlated with its overall phenolic content. More significant roles in antioxidant activity may be played by other particular phenolic molecules such flavonoids, tannins, proanthocyanidins, and amino phenolic compounds.

The maximum RP was obtained between 115 and 118 min at 30-35°C and between 10 and 13 mg/mL, as well as between 150 and 155 min at 10 and 12 mg/mL. Figure 2 D, E, and F provide a graphical depiction of this. Table 4 shows a favourable correlation between reducing power and radical scavenging activity (DPPH), but not with TPC ($p<0.05$).

**Table 4.** Pearson's correlation coefficient calculated for the response variables

| | TPC | DPPH | ABTS | RP |
|---|---|---|---|---|
| TPC | **1** | **0.352** | **0.795*** | **0.184** |
| DPPH | | **1** | **0.389** | **0.624*** |
| ABTS | | | **1** | **0.146** |
| RP | | | | **1** |

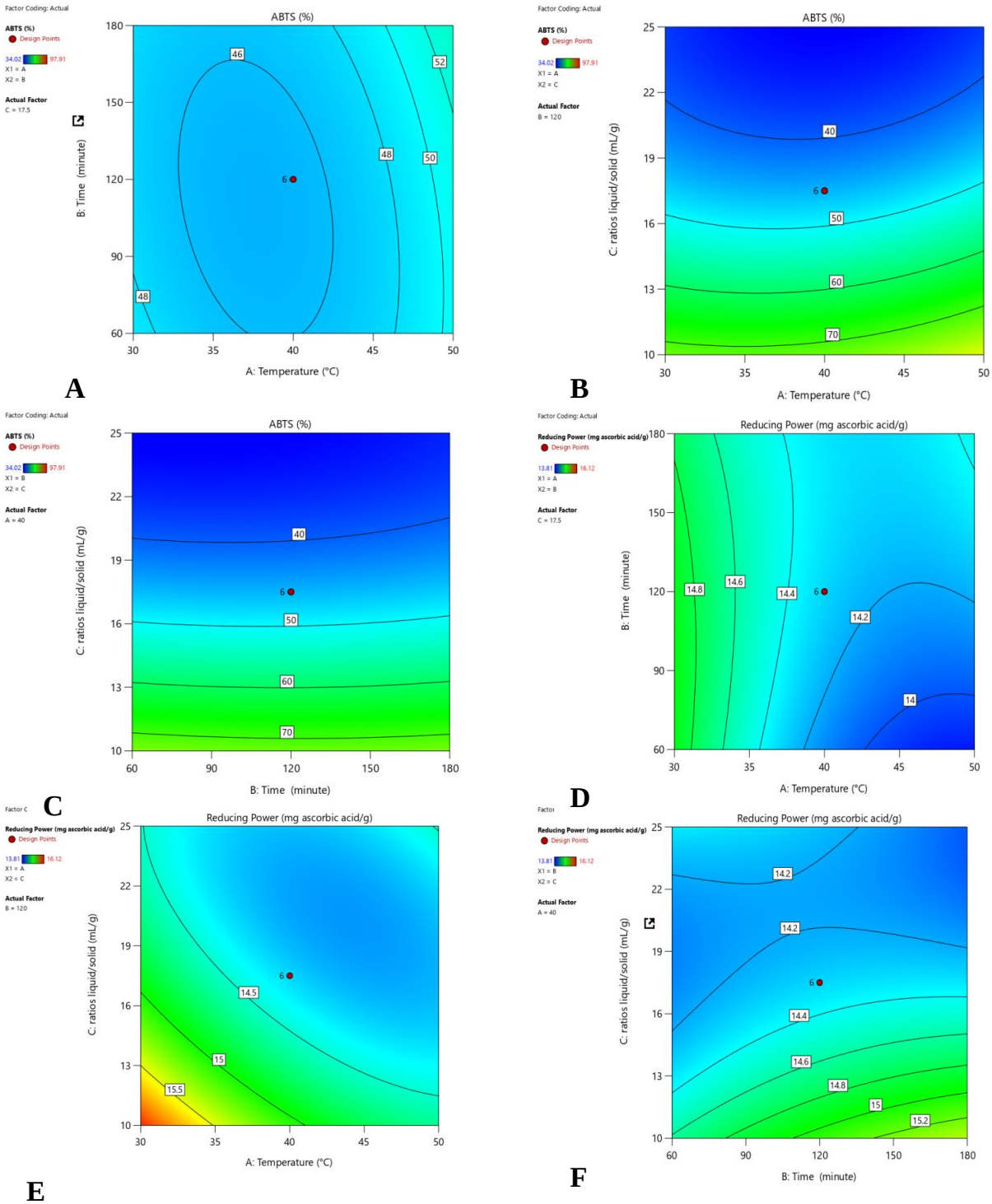


**Figure 2.** Contour plots for the effect of extraction variables on (A, B and C) ABTS% and (D, E and F) reducing power (mg ascorbic acid/g).

**Table 5.** Experimental data of the validation of predicted values at optimal extraction conditions.

| Dependent Variables | Predicted Value | Experimental Value | % CV |
|---|---|---|---|
| TPC | 6.13232 | **9.45** | **9.4** |
| DPPH | 90.5825 | **88.17** | **6.7** |
| ABTS | 58.7266 | **57.15** | **0.8** |
| RP | 14.6415 | **15.1** | **0.9** |

### *3.4. Optimization of the Extraction Parameters and Model Validation:*

Using Design Expert Software Version 10.0 (Stat-Ease, Inc., Minneapolis, MN, USA), the ideal circumstances were found by maximising the attractiveness of the replies. The greatest values for DPPH, ABTS, and RP, along with the highest TPC concentration, should ideally correspond to the maximum attractiveness. The extraction process was carried out under these optimum conditions, and responses were then identified and validated in accordance with the aforementioned approach. 40°C, 120 minutes, and 15.469 mL/g liquid/solid ratio were the ideal conditions. The desirability that was found was 0.560. The experimental results were consistent with the expected values under these ideal circumstances, with a coefficient of variation (CV) ranging from 0.8 to 9.4 (Table 5). The degree of data dispersion is indicated by this metric.

## 4.Conclusion

By extracting the phenolic compounds from the iceplant using solid-to-ethanol, it is possible to recover a significant percentage of the antioxidant activity of these compounds. Ethanolic solution (70% v/v) combined with 15.469 mL/g liquid (ethanol 70%)/solid ratio, 120 min of extraction time, and 40°C temperature encouraged the extraction of phytochemicals from the iceplant with significant phenolic compounds content and substantial antioxidant potential. The efficacy of these extracts when added to meals should be evaluated through further study.

**Compliance with Ethics Requirements**. The authors declare that they comply with the Ethics requirements of the journal. The authors declare that they have no conflicts of interest and that all procedures involving human or animal subjects (if any) comply with specific regulations and standards.

**Data availability statement.** Data available on request due to privacy/ethical restrictions.

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