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**Original Research Article**

# Extraction and Antioxidant Activity of Phenolic Compounds from *Okra* Flowers

**Sheng Geng[1], Yang Liu[2], Hanjun Ma[1] and Chungang Chen[1]***
*[1]School of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, [2]Department of Food Science, Xinyang College of Agriculture and Forestry, Xinyang 464000, China*

**For correspondence: **Email:** ccg28@126.com*

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## Abstract

***Purpose:*** *To investigate the extraction and antioxidant activity of phenolic compounds from Okra flowers.*
***Methods:*** *The phenolic compounds in Okra flowers was obtained by traditional solvent extraction method and determined by Folin-Ciocalteu (FC) method. The extraction was optimized using response surface methodology (RSM). The antioxidant activity of the obtained extract was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and reducing power assays.*
***Results:*** *The optimal extraction conditions were as follows: extraction time, 2.5 h; ethanol concentration, 59.16 %; extraction temperature, 73.91 °C; and liquid-solid ratio, 20 mL/g. The mean total phenolics yield under the optimum conditions was 40.77±0.83 mg GAE /g material, which is near the predicted value of 44.20 mg GAE /g material. The total phenolics of the extract was an effective scavenger in quenching DPPH radicals. A linear correlation between the concentration of the total phenolics extract and reducing power was observed with a correlation coefficient ($r^2$) of 0.9973.*
***Conclusion:*** *Using RSM, the extraction of total phenolics in okra flowers has been optimized. The extract exhibits a strong DPPH radical scavenging activity and reducing power, which makes it a potential functional ingredient in the food and pharmaceutical industries.*

***Keywords:*** *Okra flowers, Extraction, Response surface methodology, Phenolics, Antioxidant*

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## INTRODUCTION

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. Although ROS at physiological concentrations may be required for normal cell function, excessive production of ROS can consequently induce different kinds of serious human diseases including atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, diabetes, cancer, and some neurological disorders [1,2]. It was found that phenolic compounds could serve as antioxidants against various diseases induced by ROS [3,4].

*Okra* is an important vegetable which is widely distributed in Africa, Asia, southern European and America. Okra plays an important role in the human diet by supplying carbohydrates, minerals, and vitamins [5,6]. And its flower has been consumed as health tea and herbal medicine for hundreds years. It is reported to have many curative effects, such as antioxidant, anti-inflammatory and antitumor activities [7,8]. The most reports about *Okra* flower focused on its polysaccharide.

However, to the best of our knowledge, there are no published reports on the extraction and antioxidant activity of total phenolic compounds from *Okra* flowers, which was just the purpose of this study.

## EXPERIMENTAL

### Materials and chemicals

Flowers of *Okra* were purchased from Hebi City, Henan Province, China and identified by Dr. Guihua Xu of Henan Institute of Science and Technology. A voucher specimen (NO. CCG-1) was deposited in the Food Chemistry Lab of Henan Institute of Science and Technology. DPPH and Folin & Ciocalteu's phenol were purchased from Sigma, and DL-α-tocopherol purchased from Aladdin. All other reagents were of analytical grade.

### Extraction of phenolic compounds

The extraction was carried out by using the condensate return equipment. The sample of 1 g of the dried powder was placed in a flask and extracted with ethanol at different concentrations, different temperatures for different times, then filtered under vacuum. The filtrate was diluted to 100 mL for determining the total phenolics content.

### Determination of total phenolic yield

The total phenolic content was determined according to the Folin-Ciocalteu method [9]. Briefly, 0.2 mL of the filtrate was added to a 25 mL volumetric flask, and additional distilled water was added to make a final volume of 10 mL. A reagent blank was prepared using distilled water. Folin-Ciocalteu phenol reagent (0.5 mL) was added to the mixture and shaken vigorously. After 5 min, 5 mL of 5 % $Na_2CO_3$ solution was added with mixing. The solution was immediately diluted to 25 mL with distilled water and mixed thoroughly and then allowed to stand for 90 min. After that, the absorbance was measured at 750 nm versus the prepared blank. The total phenolic yield of the sample was expressed as gallic acid equivalents (GAE) milligrams/g raw material.

### Experimental design

Response surface methodology (RSM) with appropriate experimental designs, e.g., central composite design (CCD), has been effectively applied to optimize the intended parameters in the extraction and modification of bioactive compounds [10,11]. Response surface methodology was used to optimize experimental conditions for extraction of total phenolics from *Okra* flowers.

A three-level, four-factor, central composite design (CCD) was employed, in which 30 experiments were involved, and the total phenolics yield (Y) was used as response in evaluating the extraction (Table 1). The factors and levels studied were determined on the basis of the factorial experiments, such as extraction time (0.5, 1.5, and 2.5h), ethanol concentration (50, 75, and 100 %), temperature (30, 55 and 80 °C) and liquid-solid ratio (20, 30, 40 mL/mg). The CCD combined the vertices of a hypercube whose coordinates are given by the 2n factorial design with the "star" points. The star points were added to the factorial design to provide for estimation of curvature of the model. Six replicates (nos. 11, 13, 17, 20, 21 and 28) at the center of the design were used to allow for estimation of "pure error" sum of squares. The experiments were randomized in order to minimize the effects of unexplained variability in the observed response due to extraneous factors.

### Preparation of the extract from *Okra* flowers

The powdered flowers of okra (10 g) were extracted with 200 mL of 60 % ethanol at 74 °C for 2.5 h and then filtered under vacuum. The filtrate was collected and freeze-dried (Alpha 1-2LD plus, Christ, Germany). The obtained extract was gained for the following antioxidant assays.

### DPPH radical scavenging assay

DPPH radical scavenging assay was done according to a published method [12]. Briefly, two milliliters of DPPH solution (0.2 mmol/L in ethanol) was incubated with different concentrations of the extract, BHT (butylated hydroxytoluene). The reaction mixture was shaken and incubated in the dark for 30 min, at room temperature. The absorbance was read at 517 nm against ethanol. Controls containing ethanol instead of the antioxidant solution, and blanks containing ethanol instead of DPPH solution were also made. The inhibition of the DPPH radical (D) by the samples was calculated as in Eq 1.

$$D = \{Ac – (As – Ab)/Ac\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

### Reducing power assay

The reducing power of the sample was determined according to the published methods [13,14]. Briefy, 0.5 mL of the extract in ethanol

was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with 0.5 mL of distilled water and 0.5 mL of 0.1 % $FeCl_3$ and the absorbance was measured at 700 nm. Increased absorbance of reaction mixture indicated reducing power.

### Statistical analysis

Experimental data from CCD were analyzed by means of RSM to fit the quadratic polynomial equation with the Design Expert software (version 8.0, State-Ease, Inc., Statistics Made Easy, Minneapolis, MN). The quadratic polynomial equation is as in Eq 2.

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j \quad \text{..... (2)}$$

where $\beta_0$ was the value of the fitted response at the center point of the design, which is point (0, 0, 0). $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are the constant, linear, quadratic and cross-product regression terms, respectively.

## RESULTS

### Diagnostic checking of the fitted model

The results of the central composite design are shown in Table 1. Multiple regression analysis of the experimental data yielded the second-order polynomial stepwise equations (Eq 3).

$$Y = -50.06560 + 2.33378*X_1 + 0.13529*X_2 + 1.70130*X_3 - 11.54198*X_4 + 1.75915*10^{-3}*X_1X_2 - 8.73335*10^{-4}*X_1X_3 - 3.11905*10^{-3}*X_1X_4 + 6.70597*10^{-4}*X_2X_3 + 0.015439*X_2X_4 - 0.019884*X_3X_4 - 0.020612*X_1^2 - 1.97212*10^{-3}*X_2^2 - 0.026517*X_3^2 + 4.08541*X_4^2 \text{ …….. (3)}$$

**Table 1:** Central composite design and results

| Experiment | Coded levels | | | | Total phenolic yield (mg/g) |
|---|---|---|---|---|---|
| | Ethanol concentration (%) | Extraction temperature (°C ) | Liquid-solid ratio (mL/g) | Extraction time (h) | |
| | $X_1$ | $X_2$ | $X_3$ | $X_4$ | Y |
| 1 | 50 | 30 | 20 | 2.5 | 39.32 |
| 2 | 100 | 30 | 40 | 0.5 | 2.26 |
| 3 | 100 | 30 | 40 | 2.5 | 2.39 |
| 4 | 50 | 55 | 30 | 1.5 | 39.69 |
| 5 | 50 | 30 | 40 | 0.5 | 39.60 |
| 6 | 100 | 30 | 20 | 2.5 | 3.04 |
| 7 | 100 | 80 | 40 | 2.5 | 11.68 |
| 8 | 75 | 55 | 20 | 1.5 | 30.93 |
| 9 | 50 | 80 | 40 | 2.5 | 42.38 |
| 10 | 50 | 30 | 20 | 0.5 | 38.17 |
| 11 | 75 | 55 | 30 | 1.5 | 37.67 |
| 12 | 100 | 80 | 20 | 0.5 | 8.22 |
| 13 | 75 | 55 | 30 | 1.5 | 37.54 |
| 14 | 50 | 80 | 40 | 0.5 | 42.53 |
| 15 | 75 | 80 | 30 | 1.5 | 36.17 |
| 16 | 75 | 30 | 30 | 1.5 | 31.99 |
| 17 | 75 | 55 | 30 | 1.5 | 36.45 |
| 18 | 75 | 55 | 30 | 2.5 | 40.32 |
| 19 | 50 | 30 | 40 | 2.5 | 40.60 |
| 20 | 75 | 55 | 30 | 1.5 | 36.76 |
| 21 | 75 | 55 | 30 | 1.5 | 37.79 |
| 22 | 75 | 55 | 40 | 1.5 | 34.39 |
| 23 | 100 | 55 | 30 | 1.5 | 5.17 |
| 24 | 50 | 80 | 20 | 0.5 | 39.32 |
| 25 | 75 | 55 | 30 | 0.5 | 38.48 |
| 26 | 100 | 30 | 20 | 0.5 | 2.73 |
| 27 | 100 | 80 | 40 | 0.5 | 8.57 |
| 28 | 75 | 55 | 30 | 1.5 | 33.71 |
| 29 | 50 | 80 | 20 | 2.5 | 43.62 |
| 30 | 100 | 80 | 20 | 2.5 | 9.72 |

**Table 2:** ANOVA for the fitted model

| Source | Sum of squares | $d_f$ | Mean square | F value | Prob>F |
|---|---|---|---|---|---|
| Model | 6743.79776 | 14 | 481.6998 | 256.2845 | < 0.0001 |
| $X_1$ | 5388.52007 | 1 | 5388.52 | 2866.919 | < 0.0001 |
| $X_2$ | 98.5010599 | 1 | 98.50106 | 52.40669 | < 0.0001 |
| $X_3$ | 4.83187559 | 1 | 4.831876 | 2.57076 | 0.1297 |
| $X_4$ | 9.67061517 | 1 | 9.670615 | 5.145173 | 0.0385 |
| $X_1X_2$ | 19.3412303 | 1 | 19.34123 | 10.29035 | 0.0059 |
| $X_1X_3$ | 0.76271438 | 1 | 0.762714 | 0.405796 | 0.5337 |
| $X_1X_4$ | 0.097285 | 1 | 0.097285 | 0.05176 | 0.8231 |
| $X_2X_3$ | 0.4496999 | 1 | 0.4497 | 0.239259 | 0.6318 |
| $X_2X_4$ | 2.38372565 | 1 | 2.383726 | 1.268242 | 0.2778 |
| $X_3X_4$ | 0.6325957 | 1 | 0.632596 | 0.336567 | 0.5704 |
| $X_1^2$ | 429.966922 | 1 | 429.9669 | 228.7604 | < 0.0001 |
| $X_2^2$ | 3.93620705 | 1 | 3.936207 | 2.094227 | 0.1684 |
| $X_3^2$ | 18.2186065 | 1 | 18.21861 | 9.693062 | 0.0071 |
| $X_4^2$ | 43.2438454 | 1 | 43.24385 | 23.00754 | 0.0002 |
| Residual | 28.1932676 | 15 | 1.879551 | | |
| Lack of Fit | 16.3386043 | 10 | 1.63386 | 0.69 | |
| Pure Error | 11.8546634 | 5 | 2.370933 | | |
| Cor Total | 6771.99103 | 29 | | | |

The results of ANOVA are shown in Table 2. The Model F-value of 256.2845 implied the model is significant. There was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. The Values of "Prob > F" less than 0.0500 indicated model terms were significant. In this case $X_1$, $X_2$, $X_4$, $X_1X_2$, $X_1^2$, $X_3^2$, $X_4^2$ were significant model terms. Values greater than 0.1000 indicate the model terms were not significant. The “Lack of Fit F-value” of 0.69 implied the Lack of Fit is not significant relative to the pure error. There was a 71.23 % chance that a "Lack of Fit F-value" this large could occur due to noise. The "Pred R-Squared" of 0.9849 was in reasonable agreement with the "Adj R-Squared" of 0.9920. The above diagnostic checking of the fitted model showed the models could be used to navigate the design space.

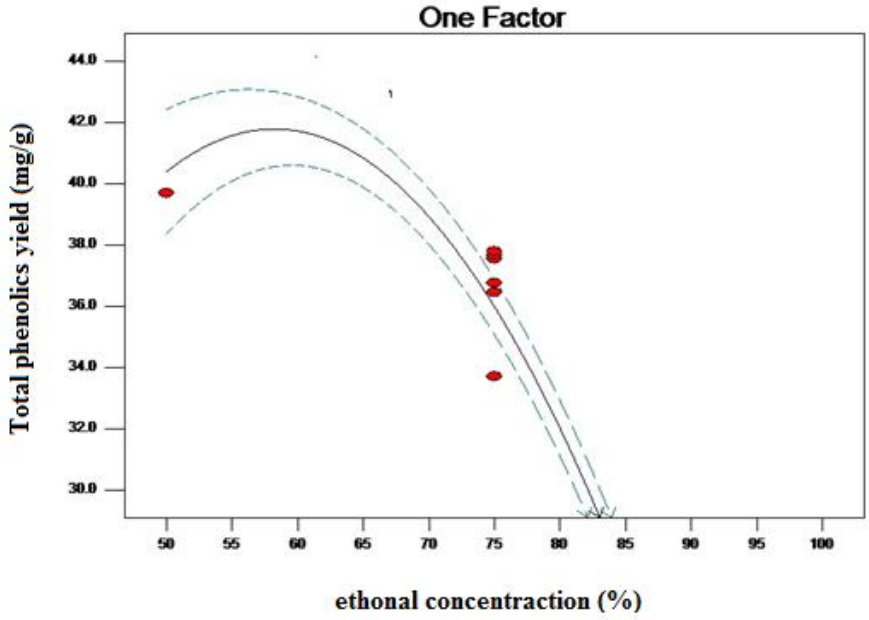



**Figure 1:** Effect of ethanol concentration on total phenolics yield

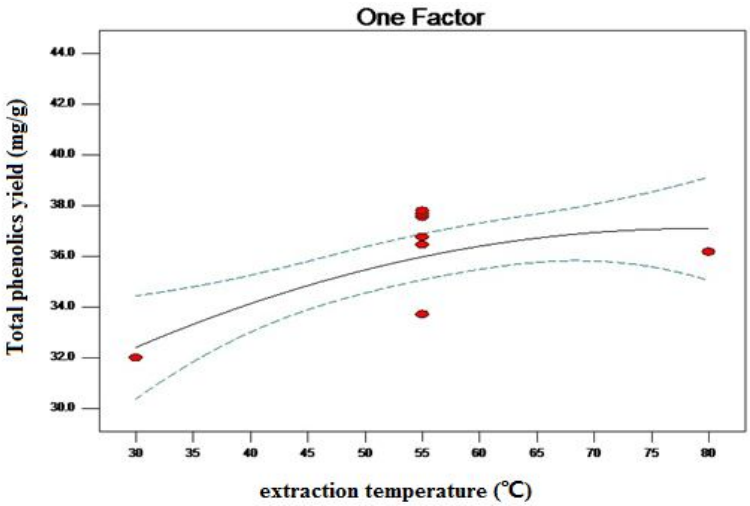



**Figure 2:** Effect of extraction temperature (B) on total phenolics yield

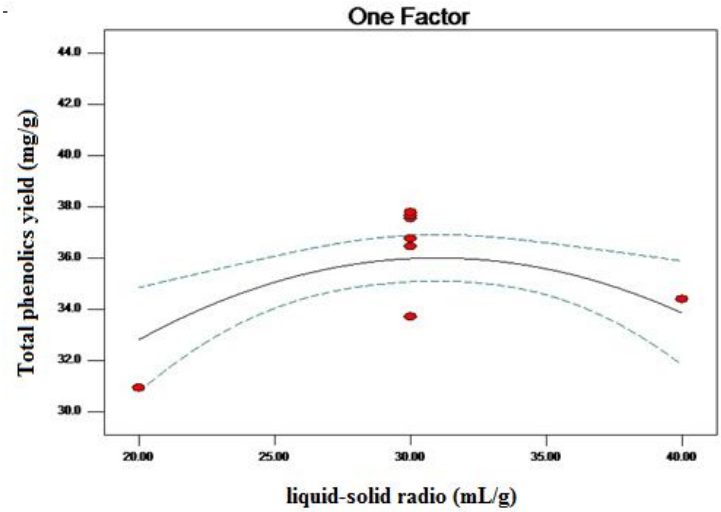



**Figure 3:** Effect of liquid-solid ratio (C) on total phenolics yield

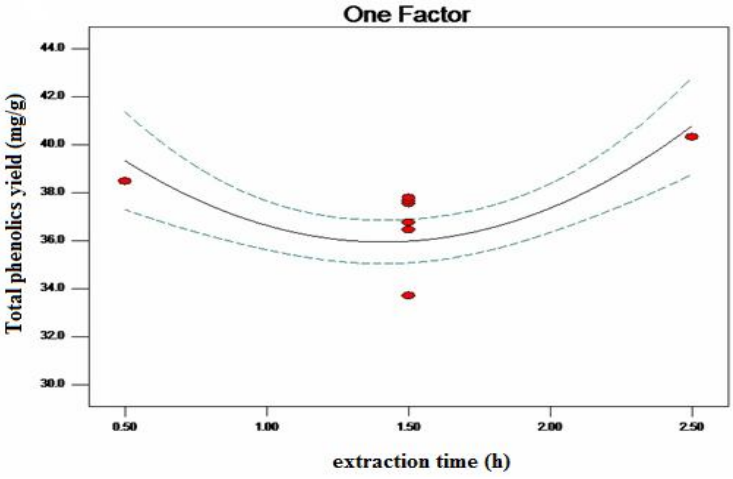
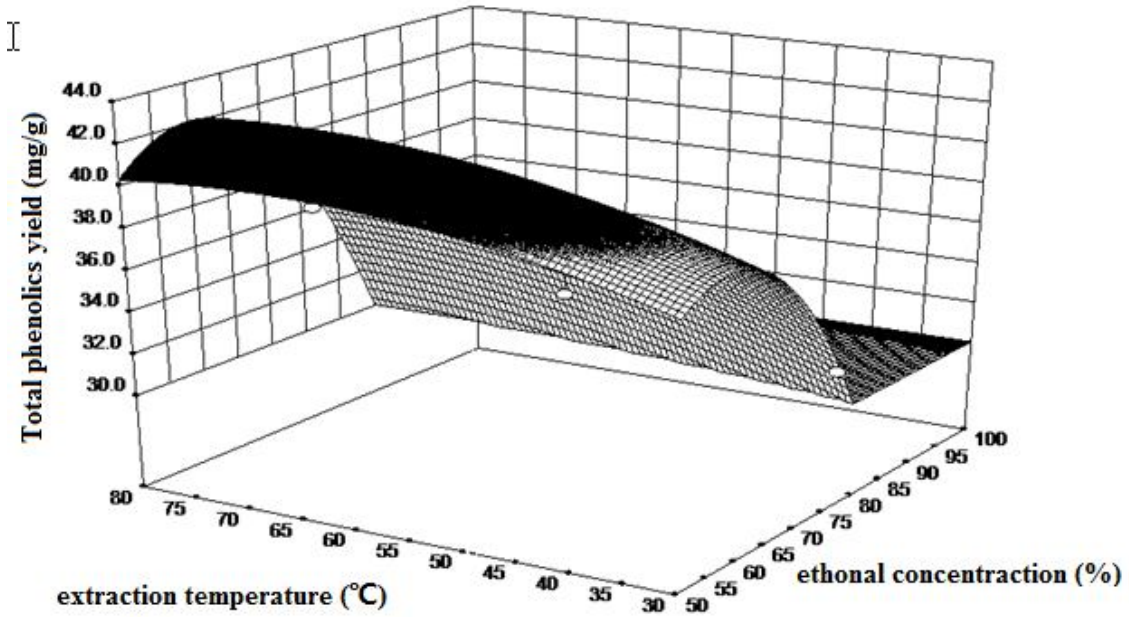



**Figure 4:** Effect of extraction temperature and ethanol concentration on total phenolics yield




**(E)**

**Figure 5:** Effect of extraction temperature and ethanol concentration on total phenolics yield

**Optimization extraction**

The effect of a series of the factors on total phenolics yield is shown Figure 1-5. The liquid-solid radio was found not significant. In order to save solvent, liquid ratio was set at 20 mL/mg, the optimum conditions were obtained by running the program of Design Expert software. The optimum conditions for independent variables and the predicted values of the responses also were presented as follows: extraction time 2.5 h, extraction temperature 73.91 °C ethanol concentration 59.16 % and liquid-solid radio 20 mL/g. The estimated values for total phenolics yield, 44.20 mg GAE /g material was obtained at those conditions. A verification experiment at the optimum condition, consisting of 3 runs, was performed and the practical yield of 40.77 ± 0.83 mg GAE /g material was obtained.

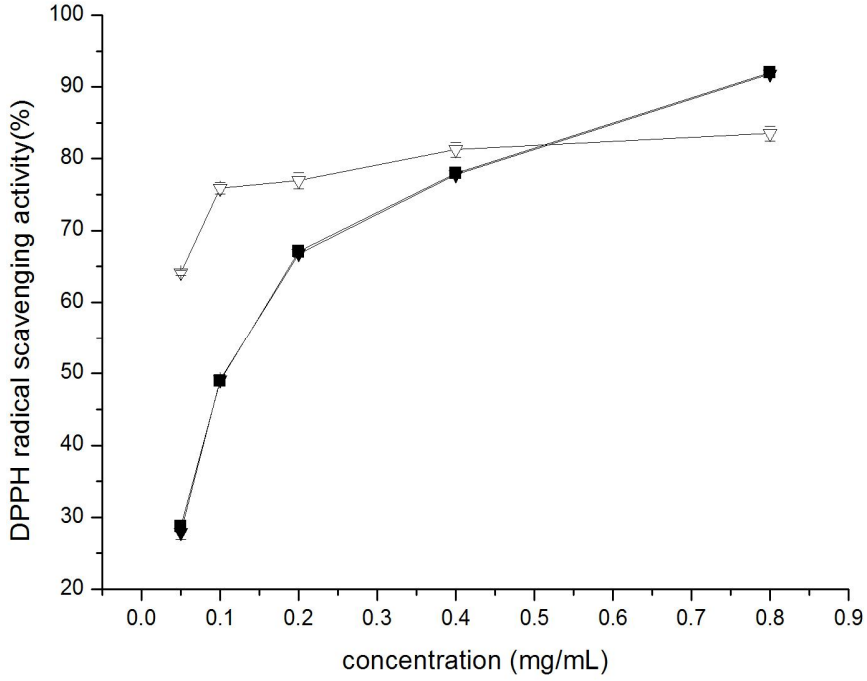



**Figure 6:** DPPH radical scavenging activity of the extract (-▽-) and BHT (-▼-)

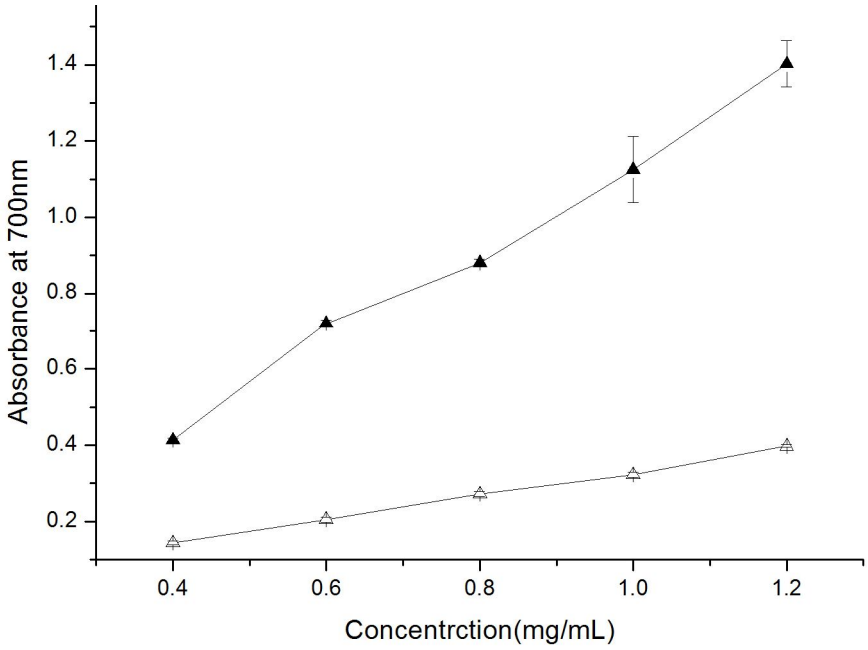



**Figure 7:** Reducing power of the extract (-△-) and Vitamin E (-▲-)

**Antioxidant activity**

In this study, high DPPH radical scavenging activity was observed by both the extract and BHT in a concentration-dependent manner (Figure 6). The DPPH radical scavenging activity of the extract was lower than that of BHT when the concentration was below 0.4 mg/mL. The DPPH scavenging activity of the extract was superior to that of BHT in the concentration range from 0.4 to 0.8 mg/mL. Figure 7 also showed the reducing power of the extract and Vitamin E. Both the samples showed some degree of reducing power. The reducing power of VE was superior to that of the extract. The reducing power of the samples linearly increased with increasing concentration and the correlative coefficient ($r^2$) of the extract and VE were 0.9973 and 0.9922, respectively.

## DISCUSSION

When many factors and interactions affect desired responses, response surface methodology (RSM) is an effective tool for optimizing the process. The basic principle behind response surface methodology (RSM) analysis is to relate the observed value (dependent variables) to process parameters (independent variables) using statistical methods, yielding a multivariate regression equation, often of second-order. RSM takes interactions into consideration and optimizes the process parameters to reasonable range, with the advantage of less number of replicates and the total time required to perform the experiments [15,16].

The relationship between the variables and responses can be better understood by examining the three-dimensional response surface plots, as shown in Figure 5, whose regression coefficients are generated from the predicted models. In this study, it was found that the performance of solid-liquid ratio is not significant. Therefore, in order to save the solvent, the solid-liquid ratio was set at 20 mL/g. Based on the above analysis, the optimum condition could be determined as extraction time of 2.5 h, extraction temperature of 73.91 °C, ethanol concentration of 59.16 % and liquid-solid radio of 20 mL/g.

The antioxidant activity of the plant extract cannot be evaluated by only a single method due to the complex nature of phytochemicals, so it is important to employ commonly accepted assays to evaluate the antioxidant activity of plant extract. Numerous antioxidant methods have been developed to evaluate antioxidant activity and to explain how antioxidants function. Of these, reducing power and DPPH assay are most commonly accepted assays for evaluating antioxidant activity. So, the DPPH radical scavenging activity and reducing power of the obtained crude total phenolics were examined. It was found that the extract was an effective scavenger of DPPH radicals and showed some degree of reducing power. Based on the results obtained, the extract, due to its high phenolic content, it can be used in functional food and medicine.

## CONCLUSION

The optimum conditions of ultrasonic-assisted extraction of total phenolics from Okra flowers can be determined by response surface methodology. The obtained extract, due to its high phenolic content, exhibits strong DPPH radical scavenging activity and reducing power. Thus, the extract is a new kind of natural antioxidant with great potential for use in the food and pharmaceuticals industries.

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