# Effect of solvent polarity and extraction time on *in vitro* antioxidant properties of *Brassica oleracea* Convar Capitata Var L. seeds

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**Abstract**: Effect of solvent polarity and extraction time on antioxidant properties of *Brassica oleracea* seeds was optimized by response surface methodology using central composite design. A significant linear negative effect of solvent polarity on total extractable components (TEC), total phenolic acids (TPA), linoleic acid reduction capacity (LARC) and hydroxyl radical scavenging capacity (HRSC) and significant positive effect on iron chelating activity (ICA), reducing power (RP) and (DPPH RSC). Linear effect of solvent polarity on total antioxidant activity (TAOA) was also found to be positive but not significant. Significant linear negative effect of extraction time was observed on ICA and HRSC. Only RP was found to be significantly increased with increase in extraction time. Quadratic effect of solvent polarity was found to be negative on TAOA, DPPH RSC and HRSC. However, quadratic effects of both variables were found to be positive on TEC, TPA, ICA and LARC. The polarity dependent increase in TAOA indicates the extraction of phytochemicals with comparatively stronger antioxidant properties in polar solvents. The decrease in antioxidant properties in response to increase in extraction time may be attributed to the time dependent loss of antioxidant activity of extracted compounds.

**Keywords**: Antioxidant properties, *Brassica oleracea* seeds, central composite design, extraction time, response surface methodology, solvent polarity.

## INTRODUCTION

Antioxidants are organic substances that are capable to prevent or minimize the damaging effects of free radicals produced naturally by oxidation in animal tissues and food materials. Free radicals are reactive oxygen and nitrogen species which cause oxidative stress in living and non living systems wherever they are produced. Antioxidants possess hydrogen donating ability due which they delay or prevent the autoxidation by i) inhibiting formation of free radicals, ii) inhibiting the initiation of per oxidation and breaking the auto-oxidative chain reaction iii) chelating metal ions that produce reactive radicals, iv) quenching $O_2^-$ radicals and reducing localized $O_2$ concentration (Wada and Ou, 2002; Huang *et al.*, 2005).

The naturally occurring antioxidant compounds such as polyphenols, phenolic acids, flavonoids, tannins and some vitamins are extracted from plants materials in different solvent using different extraction methods. The extraction efficacy of these compounds depends upon various factors such as choice of solvent, solvent concentration, solvent to solid ratio, extraction period, temperature and particle size of plant material (Pinelo *et al.*, 2005; Silva *et al.*, 2007). On the basis of their chemical nature, different phytochemicals are extracted in solvents of different polarity. The use of a single solvent does not ensure the extraction of all of the phytochemicals present in plant material. It is, therefore, adventitious to use solvents of varying polarity to ensure the complete extraction of antioxidant phytochemicals (Soong and Barlow, 2004; Marinova and Yanishlieva, 1997). The extraction efficiency of phytochemicals may also be increased by the use of combination of two or more solvents of different polarity (Jaiswal *et al.*, 2012).

*Brassica oleracea* Convar Capitata Var L. commonly known as cabbage belongs to plant family Brassicaceae. It has great nutritional and medicinal importance due to good biochemical and phytochemical composition. It has been used as traditional medicine for the treatment of cancer, obesity, dyslipidemia and gastrointestinal disorders including peptic ulcers (Cheney, 1949; Suido *et al.*, 2002; Greenly, 2005; Maritess *et al.*, 2005; Cartea *et al.*, 2010).

Previous studies showed that *B. oleracea* leaves possessed antioxidant, anticancer and antibacterial properties due to the presence of polyphenols, flavonoids, glucosinolates and their hydrolysis products (Jaiswal *et al.*, 2011). The aqueous extracts of *B. oleracea* seeds have been found to be good source of phenolic compounds and exhibit higher antioxidant potential than its leaves (Ferreres *et al.*, 2007). Various solvents such as water, methanol, ethanol, acetone, ethyl acetate, chloroform and petroleum ether and mixture of two or more of these have been used to optimize the extraction yield of antioxidant compounds from leaves of Brassica vegetables such as *B. oleracea* and cauliflower (Llorach *et al.*, 2003; Köksal and Gülçin, 2008; Jaiswal *et al.*, 2011; Anwar *et al.*, 2013). However,

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the extraction of phytochemical and antioxidant compounds from *B. oleracea* seeds has not been optimized. The present study was, therefore, focused to optimize the extraction variables including solvent polarity and extraction time on the extraction efficiency and activity of antioxidant compounds present in *B. oleracea* seed using response surface methodology. The data will contribute to the research on pharmaceutical extraction and medicinal importance of bioactive compounds present in Brassica vegetables.

## MATERIALS AND METHODS

### *Sampling*

Seeds of *B. oleracea* were purchased from local market, transported to research laboratory at Department of Chemistry, Bahauddin Zikariya University, Multan, Pakistan. The seeds were ground to fine powder and sieved through fine cloth to obtain powder of uniform particles size. The ground samples were stored in sealed containers for analysis.

### *Experimental design*

The cumulative effect of extraction time and solvent polarity on antioxidant properties of *B. oleracea* seeds was studied using response surface methodology (RSM). A central composite design (CCD) was employed to investigate the effect of two variables ($X_1$: Extraction time and $X_2$: Polarity of extracting solvents in terms of dipole moment (D), each on five levels) on antioxidant properties of *B. oleracea* seeds. CCD was arranged in such a way that it allowed to developed the appropriate empirical model. The five levels of extraction time were selected as 12, 24, 36, 48 and 60 h and those of solvent polarity as hexane 0.0, diethyl ether 2.80, ethyl acetate 4.40, methanol 5.10 and water 9.0 D.

The following equation was used for coding of selected levels of variables:

$$Xi = [\xi_i - \bar{\xi_i}/S_i]$$

$i$ = 1, 2, ……., k

Where $\xi_i$ is the specific location of independent variable, $\bar{\xi_i}$ is the centre point and $S_i$ is the scale factor *i.e.* the difference between $\xi_i$ and $\bar{\xi_i}$. $X_i$ is the coded value of an independent variable. The randomized combinations of actual and coded levels of both variables are given in table 1.

The levels variables at optimal response were searched by developing a response surface polynomial quadratic model which determined the relationship between extraction time, solvent polarity and antioxidant properties of *B. oleracea* seeds. The selected experimental model consisted of 11 points with nc = 3 centre points, nf = 4, factorial points and na-4 axial points.

### *Preparation of extracts*

Antioxidant compounds in *B. oleracea* seeds were extracted by soaking powdered sample (20g) in different solvents of various polarity (solid: solvent 1:20) at various levels of extraction time as per suggested by CCD. The suspensions were filtered through Whatman filter paper No. 1, dried extracts were obtained by evaporation of solvents and weighed to calculate total extractable contents (TEC).

$$\text{TEC}\left({}^{g}/_{100\ g\ dw}\right) = {}^{W_E}/_{W_s} \times 100$$

where $W_E$ is the weight of the extract and Ws is the weight of the sample.

### *Antioxidant analysis*

Total phenolic acid (TPA) content was determined as gram gallic acid equivalent/100 g dry weight using previously reported method (Taga *et al.*, 1984). TPA were calculated from the regression equation (TPA =Abs.at 720nm/16.488) obtained from the standard curve ($R^2$= *0.985*) of gallic acid. Total antioxidant activity (TAOA) of extract was determined as gram gallic acid equivalent /100g dry weight by previously reported phosphomolybdenum assay (Prietto *et al.*, 1999). TAOA was calculated from regression equation (TAOA = Abs.at 695nm/2.682) obtained from standard curve ($R^2$= *0.989*) of gallic acid. Reducing power of extracts was estimated according to previously reported method (Oyaizu, 1986). The ability of sample extract to chelate Fe (II) was determined by reported method (Puntel *et al.*, 2005). The linileic acid reduction capacity (LARC) of extracts was determined by ferrithiocyanate method (Osawa and Namiki, 1981). LARC was calculated as:

$$\text{LARC}\ (\%) = {}^{A_s}/_{A_c} \times 100$$

where $A_s$ is the absorbance of sample or standard and $A_c$ is the absorbance of control reaction.

Free radical scavenging capacity of seeds extract was determined by following the stable DPPH□ method reported earlier (Sánchez-Moreno *et al.*, 1998). DPPH inhibition (%) was calculated as:

$$\text{DPPH RSC}\ (\%) = {}^{(\text{Abs}_0 - \text{Abs}_{30})}/_{\text{Abs}_0} \times 100$$

where $Abs_0$ is the absorbance of control; $Abs_{30}$ is the absorbance of sample. The $IC_{50}$ values of *B. oleracea* seed exracts was determined by mixing a series of extract concentrations (0.002, 0.004,…., 0.01mg/ml) with DPPH˙ solution (3 ml) and absorbance was recorded at 517 nm after 30 min. The $IC_{50}$ value of extract was calculated by respective linear regression curve of each extract.

Hydroxyl radical scavenging activity of sample extract was assayed by previously reported method (Smirnoff and Cumbes, 1989). The scavenging activity was calculated as:

$$\text{HRSC}\ (\%) = [1 - {}^{(\text{Abs}_1 - \text{Abs}_2)}/_{\text{Abs}_0}] \times 100$$

where $Abs_1$ is the absorbance of tested sample; $Abs_2$ is absorbance of the blank and $Abs_0$ is absorbance of the control. The results were expressed as $IC_{50}$, which is the concentration of antioxidant required to scavenge the initial OH radical concentration by 50%. The $IC_{50}$ value of extract was calculated by respective linear regression curve of extract.

## STATISTICAL ANALYSIS

The results for the antioxidant properties of *B. oleracea* seeds were expressed as means of three parallel replicates. Response-surface methodology (RSM) was applied to optimized extraction parameters affecting antioxidant properties of *B. oleracea* seeds. The prediction of optimal response in range of selected levels of input variables was done by creating response surface polynomial quadratic models and regression coefficients were determined using least-squares technique (Meyers and Montgomery, 2002). The following generalized polynomial regression equation was created to define the main, linear, quadratic and interaction effects of input variables and calculate the predicted values of response:

$$Yi = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

where Yi is the predicted response, $\beta_0$ is a constant, $\beta_1$ and $\beta_2$ are the regression coefficients for linear effects, $\beta_{11}$ and $\beta_{22}$ for quadratic effects and $\beta_{12}$ for the interaction effect of input variables. The lack of fit test (F-ratio) and probability ($p \leq 0.05$) were calculated to estimate the significance of suggested model. A non-significant value of lack of fit indicates the adequacy of the model in describing the response variable (Montgomery, 2008). A model with relatively higher F-values and lower p-values is more significant (Amin and Anggoro, 2004). Only significant ($p<0.05$) terms were used in the final reduced model. The coefficient of determination ($R^2$) was calculated to determine the adequacy of the model (Weng *et al.*, 2001). The adjusted coefficient of determination ($R^2_{adj}$) was also calculated to measure the fairness of fit of the regression equation (Yang *et al.*, 2009). The reliability and precision of the experiments was checked by coefficient of variance (CV). A low value of CV indicates good precision of experiment (Thana *et al.*, 2008). The statistical software Design Expert 8.0.4.1 (Stat-Ease, Inc.) was used for response surface analysis.

For graphical optimization of independent variables, the three-dimensional plots were constructed between response and the two independent variables. The adequacy of the response surface models was verified by plotting experimental values versus those predicted by the final reduced models.

## RESULTS

The experimental values of studied parameters at various levels of solvent polarity and extraction time as per chosen by experimental design are given in table 2. TEC, TPA and TAOA, of various extracts ranged from 19.88 to 37.12, 0.03 to 0.47 and 0.17 to 0.68g/100g dw respectively. RP (Abs. at 700 nm), ICA (Abs. 510 nm), LARC (%), DPPH RSC ($IC_{50}$ mg/ml) and HRSC ($IC_{50}$ mg/ml) were found to be in range of 0.12-0.32, 0.26-0.37, 29.39-59.88, 0.009-0.018 and 0.004-0.027 respectively.

### *Response surface analysis and optimization of results*

The cumulative effect of extraction time and solvent polarity on antioxidant properties of *B. oleracea* seeds was optimized by response surface methodology (RSM). The application of RSM yielded following polynomial regression equations showing an empirical relationship between the antioxidant properties of *B. oleracea* seeds and independent variables.

$$TEC\ (g/100g\ dw) = 53.21 - 0.58X_1 - 5.85X_2 + 3.77E^{-00}X_1^2 + 0.19X_2^2 + 0.06X_1X_2$$

$$TPA\ (g/100g\ dw) = 0.046 - 2.93X_1 - 0.0419X_2 + 3.019X_1^2 - 9.542X_2^2 + 1.029X_1X_2$$

$$TAOA\ (g/100g\ dw) = 0.514 - 0.0129X_1 + 0.113X_2 + 8.05E^{-00}X_1^2 - 0.0196X_2^2 + 1.21E^{-01}X_1X_2$$

$$RP\ (Absorbance\ 700nm) = -0.144 + 0.015X_1 + 0.027X_2 - 7.6E^{-00}X_1^2 + 2.91E^{-00}X_2^2 - 1.77E^{-00}X_1X_2$$

$$ICA\ (Absorbance\ 510nm) = 0.366 - 6.20E^{-003}X_1 + 9.48E^{-00}X_2 + 1.012E^{-00}X_1^2 + 2.44E^{00}X_2^2 - 5.48E^{-004}X_1X_2$$

$$DPPH\ RSC\ (IC_{50}\ mg/ml) = 0.0198 - 3.18E^{-004}X_1 + 1.69E^{-00}X_2 + 4.07E^{-00}X_1^2 - 1.30E^{-004}X_2^2 + 1.45E^{-00}X_1X_2$$

$$HRSC\ (IC_{50}\ mg/ml) = 0.045 - 1.12E^{-003}X_1 - 2.03E^{-003}X_2 + 8.60E^{-00}X_1^2 - 1.38E^{-004}X_2^2 + 6.47E^{-00}X_1X_2$$

$$LARC\ (\%\ Inhibition) = 52.41 - 0.610X_1 - 4.187X_2 + 1.329E^{-00}X_1^2 + 0.528X_2^2 + 0.065X_1X_2$$

These polynomial regression equations include the coefficient for intercept, main effects, interaction terms and quadratic effects. The influence of each factor on response is shown by the sign and magnitude of the main effect. The main, quadratic and interaction effects of extraction time and solvent polarity on antioxidant properties of extracts were determined by analysis of variance (ANOVA) given in table 3.

The procedure of numerical optimization was followed to find out the levels of extraction time and solvent polarity at which the compounds with comparatively higher antioxidant activities would be extracted in maximum yield from *B. oleracea* seeds. The selection criteria and results for numerical optimization are presented in table 4. Optimum levels of extraction time were found to be 60, 49.63, 60, 13.14, 15.60, 55.56, 19.45 and 34.24 h and those of solvent polarity were observed as 9.00, 8.97, 5.76, 8.92, 6.34, 8.94, 8.69, 8.83 and 0.00 D for TEC, TPA, TAOA, RP, ICA, LARC, DPPH RSC and HRSC respectively.

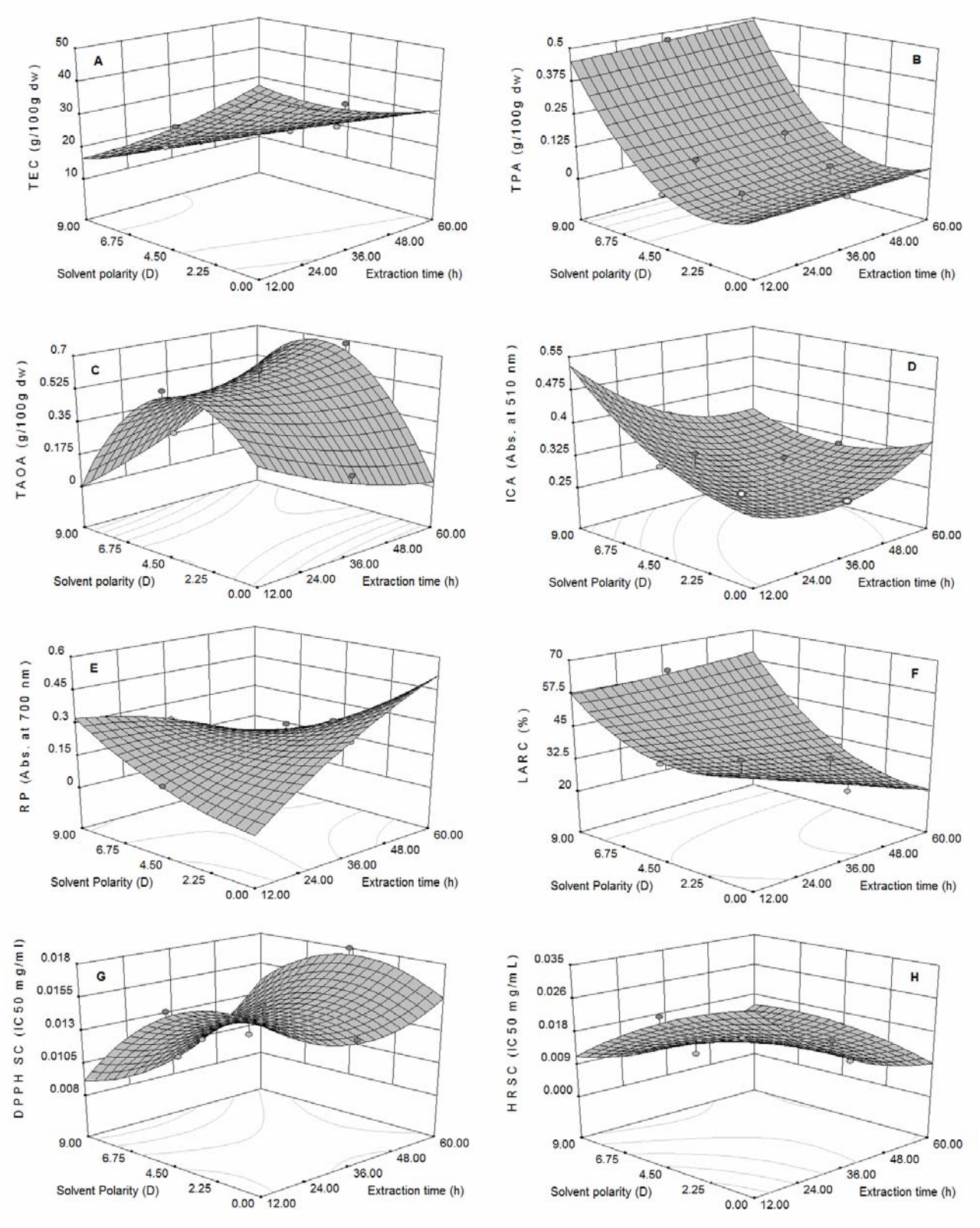
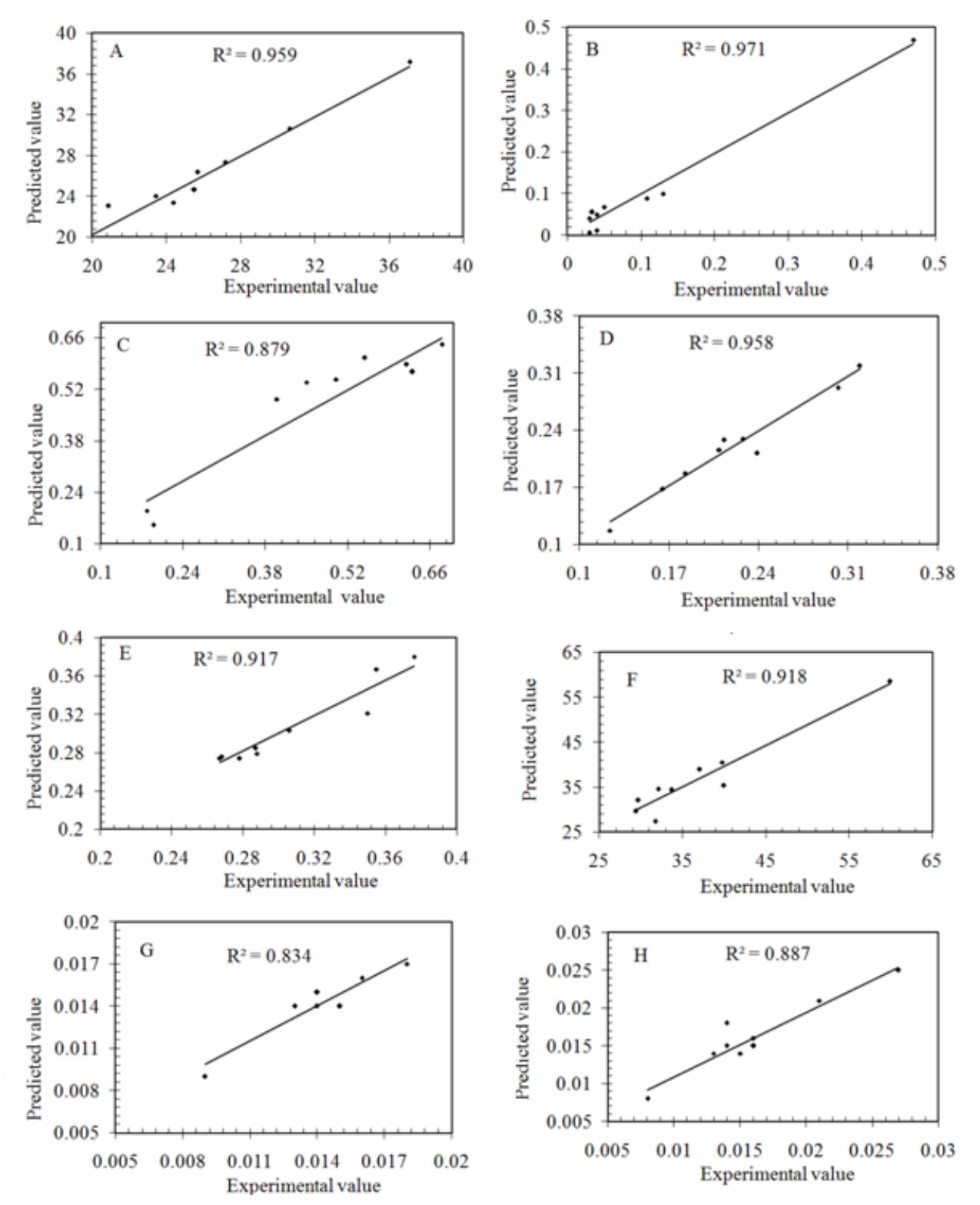



**Fig. 1A-H**: 3D response surface plot of antioxidant properties of extract of *B. oleracea* seeds at various levels of extraction conditions




A: TEC, B: TPA, C: TAOA, D: RP, E: ICA, F: LARC, G: DPPH RSC, H: HRSC

**Fig. 2A-H**: Agreement between the actual values of antioxidant properties of extract of *B. oleracea* seeds and those predicted by the response surface analysis

**Table 1**: Coded and actual levels of independent variables as per chosen by central composite design

| Experimental runs | Coded levels of variables | | Actual levels of variables | |
|---|---|---|---|---|
| | $X_1$ | $X_2$ | $\xi_1$ Extraction time (h) | $\xi_2$ Solvent polarity (D) |
| 1 | 0 | −2 | 36 | 0.00 |
| 2 | −1 | −1 | 24 | 2.80 |
| 3 | +1 | −1 | 48 | 2.80 |
| 4 | −2 | 0 | 12 | 4.40 |
| 5* | 0 | 0 | 36 | 4.40 |
| 6* | 0 | 0 | 36 | 4.40 |
| 7* | 0 | 0 | 36 | 4.40 |
| 8 | +2 | 0 | 60 | 4.40 |
| 9 | −1 | +1 | 24 | 5.10 |
| 10 | +1 | +1 | 48 | 5.10 |
| 11 | 0 | +2 | 36 | 9.00 |

| Coded levels | −2 | −1 | 0 | +1 | +2 |
|---|---|---|---|---|---|
| $\xi_1$ : Extraction time (h) | 12 | 24 | 36 | 48 | 60 |
| $\xi_2$ : Solvent polarity (D) | 0.00 | 2.80 | 4.40 | 5.10 | 9.00 |

**Table 2**: Antioxidant profile of *B. oleracea* seed extracts at selected levels of solvent polarity and extraction time.

| | $T_{ext}$ (h)[a] | $P_s$ (D) | TEC (g/100g dw) | TPA (g/100g dw) | TAOA (g/100g dw) | RP (Abs. at 700 nm) | ICA (Abs. at 510 nm) | DPPH RSC ($IC_{50}$ mg/mL) | HRSC ($IC_{50}$ mg/mL) | LARC (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36 | 0.00 | 37.12 | 0.03 | 0.19 | 0.32 | 0.27 | 0.014 | 0.016 | 29.64 |
| 2 | 24 | 2.80 | 30.66 | 0.03 | 0.45 | 0.16 | 0.28 | 0.014 | 0.021 | 39.94 |
| 3 | 48 | 2.80 | 25.68 | 0.04 | 0.40 | 0.30 | 0.26 | 0.014 | 0.015 | 31.71 |
| 4 | 12 | 4.40 | 27.18 | 0.04 | 0.62 | 0.12 | 0.35 | 0.016 | 0.027 | 39.77 |
| 5 | 36 | 4.40 | 25.5 | 0.03 | 0.63 | 0.20 | 0.26 | 0.015 | 0.016 | 33.66 |
| 6 | 36 | 4.40 | 25.5 | 0.03 | 0.63 | 0.20 | 0.26 | 0.015 | 0.016 | 33.66 |
| 7 | 36 | 4.40 | 25.5 | 0.03 | 0.63 | 0.20 | 0.26 | 0.015 | 0.016 | 33.66 |
| 8 | 60 | 4.40 | 24.38 | 0.05 | 0.68 | 0.31 | 0.30 | 0.018 | 0.014 | 29.39 |
| 9 | 24 | 5.10 | 23.44 | 0.20 | 0.50 | 0.18 | 0.35 | 0.013 | 0.014 | 37.04 |
| 10 | 48 | 5.10 | 20.88 | 0.23 | 0.54 | 0.23 | 0.26 | 0.014 | 0.013 | 32.06 |
| 11 | 36 | 9.00 | 19.88 | 0.47 | 0.17 | 0.22 | 0.37 | 0.009 | 0.004 | 59.88 |

[a]$T_{ext}$ : Extraction time, $P_s$ : Solvent polarity

**Table 3**: Analysis of variance (ANOVA), coefficient of variation (CV) and correlation coefficient ($R^2$) as calculated by response surface model for antioxidant properties of extracts of *B. oleracea* seeds

| | $\beta_o$ | $\beta_1$ | $\beta_2$ | $\beta_{12}$ | $\beta_1^2$ | $\beta_2^2$ | CV | $R^2$ | Adjusted $R^2$ | Adequate precision |
|---|---|---|---|---|---|---|---|---|---|---|
| TEC | | | | | | | | | | |
| *F*-value | 11.08 | 0.78 | 50.44 | 0.93 | 1.74 | 6.22 | 6.95 | 0.92 | 0.84 | 12.70 |
| *p*-value | 0.008 | 0.41 | 0.0009 | 0.38 | 0.243 | 0.05 | | | | |
| TPA | | | | | | | | | | |
| *F*-value | 33.37 | 6.23 | 106.4 | $8.99E^{-003}$ | $3.77E^{-003}$ | 50.52 | 34.69 | 0.97 | 0.94 | 19.94 |
| *p*-value | 0.0008 | 0.65 | 0.0001 | 0.92 | 0.95 | 0.0009 | | | | |
| TAOA | | | | | | | | | | |
| *F*-value | 7.28 | 0.34 | 0.11 | 0.40 | 0.35 | 28.16 | 17.48 | 0.87 | 0.75 | 7.64 |
| *p*-value | 0.0241 | 0.5834 | 0.7505 | 0.5557 | 0.578 | 0.0032 | | | | |
| RP | | | | | | | | | | |
| *F*-value | 23.11 | 27.12 | 18.12 | 10.34 | 9.27 | 18.15 | 7.34 | 0.95 | 0.91 | 17.10 |
| *p*-value | 0.0018 | 0.0034 | 0.0080 | 0.0236 | 0.0286 | 0.0080 | | | | |
| ICA | | | | | | | | | | |
| *F*-value | 10.85 | 10.72 | 23.19 | 0.92 | 15.28 | 11.92 | 5.51 | 0.91 | 0.83 | 8.65 |
| *p*-value | 0.010 | 0.022 | 0.004 | 0.381 | 0.011 | 0.018 | | | | |
| LARC | | | | | | | | | | |
| *F*-value | 11.22 | 6.14 | 33.14 | 0.30 | 0.060 | 12.75 | 9.52 | 0.91 | 0.83 | 12.15 |
| *p*-value | 0.0095 | 0.0559 | 0.0022 | 0.6072 | 0.8157 | 0.0160 | | | | |
| DPPH RSC | | | | | | | | | | |
| *F*-value | 7.20 | 2.28 | 10.00 | 0.16 | 6.22 | 8.57 | 7.29 | 0.87 | 0.75 | 10.56 |
| *p*-value | 0.024 | 0.191 | 0.025 | 0.704 | 0.054 | 0.032 | | | | |
| HRSC | | | | | | | | | | |
| *F*-value | 9.34 | 16.55 | 9.62 | 0.82 | 7.04 | 2.42 | 12.97 | 0.90 | 0.80 | 11.37 |
| *p*-value | 0.014 | 0.009 | 0.026 | 0.407 | 0.045 | 0.180 | | | | |

**Table 4**: Optimum levels of input variables to achieve the desired goals of response variables with maximum desirability

| Variables | Goal | Lower limit | Upper limit | Optimum levels | | | Desirability |
|---|---|---|---|---|---|---|---|
| | | | | $X_1$ | $X_2$ | Y | |
| Extraction time (h) | in range | 12 | 60 | | | | |
| Solvent polarity (D) | in range | 0.00 | 9.00 | | | | |
| TEC (g/100g dw) | maximize | 19.88 | 37.12 | 60 | 9.00 | 27.55 | 0.67 |
| TPA (g/100g dw) | maximize | 0.03 | 0.47 | 49.63 | 8.97 | 0.47 | 1.00 |
| TAOA (g/100g dw) | maximize | 0.17 | 0.68 | 60 | 5.76 | 0.67 | 0.99 |
| RP (Absorbance 700nm) | maximize | 0.12 | 0.31 | 13.14 | 8.92 | 0.31 | 1.00 |
| ICA (Absorbance 510nm) | maximize | 0.26 | 0.37 | 15.60 | 6.34 | 0.39 | 1.00 |
| LARC (% Inhibition) | maximize | 29.39 | 59.88 | 55.56 | 8.94 | 60 | 1.00 |
| DPPH RSC ($IC_{50}$ mg/mL) | minimize | 0.009 | 0.018 | 19.45 | 8.69 | 0.009 | 1.00 |
| HRSC ($IC_{50}$ mg/mL) | minimize | 0.008 | 0.027 | 34.24 | 8.83 | 0.008 | 1.00 |

## DISCUSSION

In present study, the effect of polarity of extracting solvents and duration of extraction on antioxidant activity of *B. oleracea* seeds extracts was determined using RSM. The antioxidant activity was determined in terms of TPA, TAOA, RP, ICA, LARC, DPPH RSC and HRSC. The prediction of an optimum level of each of the independent variables was carried out by using central composite design (CCD). RSM indicated that the relationship between extraction variables and the antioxidant profile of extracts could be explained by suggested models. The lack of fit (*F-value*), probability (*p-value*), coefficient of determination ($R^2$) and adjusted $R^2$ were calculated to measure the significance, adequacy, validity and goodness-of-fit of the response surface model and regression equation (Rodrigues *et al.*, 2008; Yang *et al.*, 2009). *F*-value and *p*-values showed that extraction time has linear negative effect on TEC, TPA, TAOA, ICA, LARC, DPPH RSC and HRSC which was found to be significant on ICA, LARC and HRSC. Quadratic effect of extraction time was found to be positive on each of the studied parameter except RP but was found to be significant on ICA and HRSC. Extraction time has significant linear positive and quadratic negative effect on RP. Solvent polarity was found to have linear negative effect on TEC, TPA, LARC and HRSC and linear positive effect on TAOA, ICA, RP and DPPH RSC. The linear effects of solvent polarity were found to be significant on each parameter except TAOA. Quadratic effect of solvent polarity was found to be negative on TAOA, DPPH RSC and HRSC and positive on rest of the parameters. Quadratic effect of solvent polarity was found to be significant on each parameter except TEC and HRSC. Interaction effect of both variables was found to be positive but not significant on each parameter except RP which was negative and significant. The values of $R^2$ showed that the suggested model can explain more than 90% of variability in TEC, TPA, RP, ICA, LARC and HRSC and more than 85% variability in TAOA and DPPH RSC. The values of adjusted $R^2$ for these responses (0.7561-0.9418) also advocate the significance of the model. Relatively low values of CV (5.51-12.97%) and high values of adequate precision (7.64-17.11) showed better precision and reliability for TEC, ICA, RP, LARC, DPPH RSC and HRSC while relatively high values of CV (17.10 and 34.69%) and low values of adequate precision (7.64 and 19.94) showed less precision for TAOA and TPA respectively. Three dimensional (3D) response surface plots were drawn to show the main, quadratic and interaction effects of extraction time and solvent polarity on antioxidant properties (fig.1A-H).

The experimental and predicted values of responses were found to be correlated with the studied parameters with high values of $R^2$ (0.834-0.971) which proves the applicability of the proposed model to optimize the effect of extraction conditions on antioxidant properties of the *B. oleracea* seeds extracts with greater accuracy.

The present study indicates that the extraction of antioxidant phytochemicals from plant material particularly *B. oleracea* seeds is significantly affected by the polarity of extracting solvent and duration of extraction. The study also indicates that *B. oleracea* seed contain phytochemicals extracted in non-polar solvents but show relatively low antioxidant activity. It also contain some phytochemicals which are extracted in polar solvents in low yield but possess good antioxidant properties. The decrease in antioxidant properties in response to increase in extraction time does not agree with the prolonged extraction durations for extraction of antioxidant phytochemicals.

## CONCLUSION

The linear decrease in TEC and TPA and increase in TAOA in response to increase in solvent polarity indicate the presence of non polar phytochemical compounds with relatively low antioxidant activity in *B. oleracea* seeds. The polarity dependent increase in TAOA also indicates that the phytochemicals extracted from *B. oleracea* seeds in high polarity solvents are comparatively stronger antioxidants. The decrease in antioxidant properties in

response to increase in extraction time may be attributed to time dependent degradation of antioxidant compounds. The study, therefore, suggests the use of a mixture of polar and non polar solvents in suitable proportions for maximum extraction of antioxidant compounds from *B. oleracea* seeds and other plant materials. The study would be a significant contribution in the in the field research on extraction and analysis of antioxidant phytochemicals from plant materials.

## ACKNOWLEDGEMENTS

The authors are grateful to Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan for providing the laboratory facilities throughout the experimental work.

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