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Short communication

Optimization of extraction yield and antioxidant properties of *Brassica oleracea* Convar Capitata Var L. leaf extracts

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ABSTRACT

Effects of extraction time and solvent polarity on antioxidant properties of *Brassica oleracea* leaves were optimized by response surface methodology using a central composite design. Five extraction times (12, 24, 36, 48 and 60 h) and solvent polarities (dipole moment – hexane: 0.0, diethyl ether: 2.80, ethyl acetate: 4.40, methanol: 5.10 and water: 9.0 D) were selected for optimization. Response surface analysis of data showed a significant increase ($p < 0.05$) in extract yield and antioxidant potential, based on total phenolic acids, reducing abilities and free radical scavenging capacities, in response to an increase in extraction time and solvent polarity. The optimal response was obtained using relatively polar solvents (4.40–9.00 D) and prolonged extraction times (50–60 h). This suggests that most of the phytochemical constituents of *B. oleracea* leaves are polar and possess strong antioxidant potential.

1. Introduction

Antioxidants are the naturally occurring as well as the synthetic organic compounds capable of preventing or minimizing the damaging effects of free radicals (Lobo, Patil, Phatak, Chandra, et al., 2010; Nimse & Pal, 2015; Sisein, 2014). The antioxidants perform their function by various mechanisms including inhibition of free radical formation, inhibition of free radical chain reaction, chelating free radical producing metal ions and reducing the localized O_2 concentration by quenching O_2^- radicals (Huang, Ou, & Prior, 2005; Nimse & Pal, 2015; Wada & Ou, 2002).

The naturally occurring antioxidant phytochemicals such as polyphenols, phenolic acids, flavonoids, anthocyanins, proanthocyanidins, ascorbic acid and tocopherols can be utilized best as a part of our balanced diet to strengthen our antioxidant defense system against free radical damage. However, the researchers need to extract these compounds from plant materials to analyze their antioxidant potential for the preparation of natural antioxidant based pharmaceutical formulations. These Antioxidant compounds can be extracted from plant materials using different solvents and extraction methods. The extract yield of these methods depends on factors such as choice of solvent, solvent concentration, the solvent to solid ratio, extraction period, extraction temperature and particle size of the plant material (Pinelo, Del Fabbro, Manzocco, Nuñez, & Nicoli, 2005; Silva, Rogez, & Larondelle, 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, required to use a combination of solvents of varying polarity to obtain maximum extract yield of phytochemicals with good antioxidant potential for the analytical and preparatory purpose (Jaiswal, Abu-Ghannam, & Gupta, 2012).

Brassica oleracea L, commonly known as cabbage, possesses antioxidant, anticancer and antibacterial properties due to the presence of polyphenols and their hydrolysis products (Jaiswal, Rajauria, Abu-Ghannam, & Gupta, 2011). Various solvents, such as water, methanol, ethanol, acetone, ethyl acetate, chloroform and petroleum ether have been used previously to optimize the extraction of antioxidant phytochemical compounds from Brassica vegetables (Ahmed, Rao, Ahemad, & Ibrahim, 2012; Anwar et al., 2013). However, no data are available regarding the cumulative effect of solvent polarity and extraction time on antioxidant potential of extracts from Brassica vegetables. The present study, therefore, focused on optimizing the effect of solvent polarity and extraction time on the extraction yield and activity of antioxidant compounds present in *B. oleracea* leaves using response surface methodology (RSM).

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2. Material and methods

2.1. Chemicals and reagents

1,10-Phenanthroline, 2,2-diphenyl-1-picrylhydrazyl, tris-hydrochloric acid, Folin-Ciocalteu reagent, potassium ferricyanide, ammonium thiocyanate, ammonium molybdate, gallic acid, linoleic acid, hydrogen peroxide, ferrous sulfate and ferric chloride were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen Germany). Sodium carbonate (Na_2CO_3), potassium dihydrogen phosphate, trichloroacetic acid, ferric sulfate, sodium chloride, salicylic acid, Sulfuric acid, hexane, diethyl ether, ethyl acetate and methanol were purchased from Merck Millipore (KGaA, Darmstadt, Germany). All the chemicals and solvents were of analytical grade and used without any purification.

2.2. Samples

B. oleracea leaves were purchased from local market, washed in distilled water, dried under shade at room temperature (25 ± 5 °C) and ground to fine powder using an electric grinder (National Juicer Blender & Grinder JPN 176, Japan) at low speed (1000 rpm) to avoid temperature fluctuation. The powder was sieved through a fine cloth containing about 60 meshes per inch to obtain the small particle size (< 250 μm). The ground samples were stored in air tight containers for further analysis.

2.3. Experimental design for the extraction of antioxidants

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 the extraction yield and antioxidant properties of extracts from *B. oleracea* leaves. The five extraction time were 12, 24, 36, 48, and 60 h and solvent polarities were 0.0 (hexane), 4.40 (diethyl ether), 2.80 (ethyl acetate), 5.10 (methanol) and 9.0 D (water). The following generalized equation was used for coding of selected levels of variables:

$$Xi = [\xi_i - \xi_i^- / S_i] \quad (1)$$

where, $i = 1, 2, \ldots, k$, ξ_i is the specific location of the independent variable, ξ_i^- is the center point and S_i is the difference between ξ_i and ξ_i^-. X_i is the coded value of an independent variable. The randomized combinations of actual and coded levels of input variables as chosen by CCD are shown in Table 1. The selected experimental model consisted of 11 points with nc = 3 center points, nf = 4 factorial points and na = 4 axial points.

B. oleracea leaf samples (20 g) were extracted in solvents of different polarity (solid/solvent 1:20) for different times according to the experimental design. The solvents were evaporated to dryness, extracts were weighed and total extract yield (TEY) was calculated as:

$$TEY\,(\text{g}/100\text{ g dry wt.}) = weight\ of\ extract / weight\ of\ sample \times 100 \quad (2)$$

2.4. Antioxidant analysis

Total phenolic acids (TPA) content of extracts was estimated by Folin-Ciocalteu method described earlier (Nawaz, Shad, & Batool, 2013). TPA content was calculated as gallic acid equivalent g/100 g dry wt. using the regression equation obtained from the standard curve of gallic acid ($R^2 = 0.985$).

Total antioxidant activity (TAOA) was determined by phosphor-molybdenum assay described earlier (Prietto, Pineda, & Aquilar, 1999). TAOA was calculated as gallic acid equivalent g/100g dry wt. using regression equation obtained from the standard curve of gallic acid ($R^2 = 0.989$).

The linoleic acid reduction capacity (LARC), reducing power (RP) and iron chelating activity were determined using previously described methods (Osawa & Namiki, 1981; Oyaizu, 1986; Puntel, Nogueira, & Rocha, 2005). Free radical scavenging capacity of extracts was determined as DPPH radical scavenging capacity (DPPH RSC) and hydroxyl radical scavenging capacity (HRSC) using the previously described methods (Smirnoff & Cumbes, 1989; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). DPPH RSC and HRSC were expressed in terms of IC_{50} values (concentration of extracts needed for 50% scavenging of radicals).

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		
	X1	X2	ξ1 Extraction time (h)	ξ2 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
ξ_1: Extraction time (h)	12	24	36	48	60
ξ_2: Solvent polarity (D)	0.00	2.80	4.40	5.10	9.00

* Central points.

2.5. Statistical analysis

The results for the antioxidant properties of *B. oleracea* leaf extracts were expressed as means of three parallel replicates. Response surface models were created for the prediction of change in response and optimization of independent variables. The generalized polynomial model for predicting the variation in the response variable is given below:

$$Yi = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (3)$$

where Yi is the predicted response, β_o is a constant, β_1 and β_2 are the regression coefficients for the main variable effects, β_{11} and β_{22} are quadratic effects and β_{12} is the interaction effect of independent variables. The significance, adequacy and reliability of the suggested model were determined by analysis of variance (ANOVA). The development of experimental design, data analysis and optimization procedures were performed using the statistical software Design Expert 8.0.4.1 (Stat-Ease, Inc.).

3. Results and discussion

In present study the antioxidant potential of *B. oleracea* leaf extracts was determined and the results are presented in Table 2. One way analysis of variance (ANOVA) showed significant variation ($p \leq 0.05$) in observed values of each parameter at various combinations of solvent polarity and extraction time. The results indicated that extraction in high polarity solvents (> 4.40 D) for more than 36 h gives a comparatively higher value of TEY, TPA, and antioxidant activity.

The time-dependent increase in extract yield and antioxidant properties suggests prolonged extraction time for complete extraction of phytochemical antioxidants. The increase in TEY and phenolic acids content in response to an increase in solvent polarity suggests that *B.*