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ORIGINAL ARTICLE

Optimization protocol for the extraction of antioxidant components from *Origanum vulgare* leaves using response surface methodology

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Abstract In the present work, the response surface methodology (RSM) based on a central composite rotatable design (CCRD), was used to determine optimum conditions for the extraction of antioxidant compounds from *Origanum vulgare* leaves. Four process variables were evaluated at three levels (31 experimental designs): methanol (70%, 80%, and 90%), the solute:solvent ratio (1:5, 1:12.5, 1:20), the extraction time (4, 10, 16 h), and the solute particle size (20, 65, 110 micron). Using RSM, a quadratic polynomial equation was obtained by multiple regression analysis for predicting optimization of the extraction protocol. Analysis of variance (ANOVA) was applied and the significant effect of the factors and their interactions were tested at 95% confidence interval. The antioxidant extract (AE) yield was significantly influenced by solvent composition, solute to solvent ratio, and time. The maximum AE was obtained at methanol (70%), liquid solid ratio (20), time (16 h), and particle size (20 micron). Predicted values thus obtained were closer to the experimental value indicating suitability of the model. Run 25 (methanol:water 70:30; solute:solvent 1:20;

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extraction time 16 h and solute particle size 20) showed highest TP contents (18.75 mg/g of dry material, measured as gallic acid equivalents) and DPPH radical scavenging activity (IC_{50} 5.04 μg/mL). Results of the present study indicated good correlation between TP contents and DPPH radical scavenging activity. Results of the study indicated that phenolic compounds are powerful scavengers of free radical as demonstrated by a good correlation between TP contents and DPPH radical scavenging activity.

1. Introduction

The *Origanum* is one of the main genuses of the family *Lamiaceae* and comprises more than 38 species of annual, perennial and shrubby herbs, most of which are native to or restricted to the eastern part of the Mediterranean area, Europe, Asia and North Africa (Hussain et al., 2011). This genus includes some important culinary herbs and medicinal plants, including *Origanum vulgare* L. (common name 'oregano') (Esen et al., 2007). The use of the *O. vulgare* plant for the treatment of various diseases was in practice, being antiseptic and stimulant (Özcan and Chalchat, 2009). Besides their commercial importance, such plants have been used, for long, as condiments and spices for foods like salads, soups, sausages and meats (Hussain et al., 2011; Matsuura et al., 2003). The antioxidant and other biological properties of the *Origanum* extracts and essential oil have recently been of great interest in both academia and food industries because of their antioxidant and radical scavenging potentials (Hussain et al., 2011; Matsuura et al., 2003).

Quantity and quality of plant extracts are dependent on extraction protocol (Hussain et al., 2012; Sultana et al., 2009). Many factors including type of solvent, the temperature, the pH, the number of extraction steps, liquid-to-solid ratio and the particle size of the solute contribute to the efficacy of the extraction process (Mafart and Béliard, 1992). The extraction parameters selected must be ensured to complete extraction of the compounds of interest, and it must avoid their chemical modification (Wu et al., 2011). Extraction techniques are employed taking into account the chemistry and uneven distribution of phenolic antioxidants in the plant matrix. Depending upon the stability and nature of the phenolic compounds different extracting solvent/procedures are used for extraction purposes. Polar solvents like methanol, ethanol are frequently employed for the recovery of polyphenols from different plant matrices (Anwar et al., 2009a; Sultana et al., 2007). However, a single solvent may not be able to extract maximum phenolic compounds from all types of plant materials. Combinations of aqueous-organic solvents are more efficient in recovering antioxidants than corresponding pure solvents (Sultana et al., 2009).

In classical optimization experiments only one factor is variable at a time and this method is called as one-factor-at-a-time approach. This technique is tedious, expensive, consuming and failed to elaborate the interaction effects between variables. Response surface methodology (RSM) is a useful method to evaluate the effects of multiple factors and their interactions on one or more response variables. RSM can effectively be used to find a combination of factor levels that produce an optimum response. One of the main advantages of this method is that it generally requires fewer experimental runs than what is needed in traditional full factorial designs, while providing statistically acceptable results (Silva et al., 2007). It has been used for optimization of various food processing methods such as milling, extraction, and fermentation (Baş and Boyacı, 2007; Quanhong and Caili, 2005).

The objective of this study was to use the RSM approach to optimize the conditions (solvent concentration, solute:solvent ratio, extraction time and solute particle size) for the extraction of antioxidant compounds from *O. vulgare* leaves and to measure total phenolic contents and free radical scavenging capacity of obtained extracts.

2. Materials and methods

2.1. Collection of sample

Leaves of the *Oreganum vulgare* (*O. vulgare*) plant were collected from the Gattwala Forest Park, Faisalabad, Pakistan. The specimens were further identified and authenticated from Dr. Muhammad Naeem, Department of Botany, Government College University Faisalabad (Voucher No. 24953, University of Agriculture Faisalabad, Pakistan). Samples were dried at room temperature and ground into a semi powder using (LG BL 999SP) and finally passed from the vibratory sieve shaker (Octagon sieve (OCT-DIGITAL 4527-01) to gain different mesh sizes (20, 65 and 110 micron). These samples were stored in air tight polythene bags at 4 °C for the extraction process.

2.2. Chemicals and reagents

Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and Folin–Ciocalteu reagent were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and solvents used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise.

2.3. Optimization of parameters for extraction of bioactive components

A protocol for the extraction of bioactive components from *O. vulgare* leaves was established by response surface methodology (RSM) which was employed to determine the best combination of variables for optimum extraction yield and antioxidant activity. RSM was used to analyze the influence of four extraction process variables, namely, the methanol/water ratio, solute/solvent ratio, the extraction time, and the solute particle size, on the yield of antioxidant extracts.