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Research article

Effect of Korean Red Ginseng extraction conditions on antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content: optimization using response surface methodology

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ABSTRACT

Background: Extraction conditions greatly affect composition, as well as biological activity. Therefore, optimization is essential for maximum efficacy.
Methods: Korean Red Ginseng (KRG) was extracted under different conditions and antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content evaluated. Optimized extraction conditions were suggested using response surface methodology for maximum antioxidant activity and extraction yield.
Results: Analysis of KRG extraction conditions using response surface methodology showed a good fit of experimental data as demonstrated by regression analysis. Among extraction factors, such as extraction solvent and extraction time and temperature, ethanol concentration greatly affected antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content. The optimal conditions for maximum antioxidant activity and extraction yield were an ethanol concentration of 48.8%, an extraction time 73.3 min, and an extraction temperature of 90°C. The antioxidant activity and extraction yield under optimal conditions were 43.7% and 23.2% of dried KRG, respectively.
Conclusion: Ethanol concentration is an important extraction factor for KRG antioxidant activity and extraction yield. Optimized extraction conditions provide useful economic advantages in KRG development for functional products.

1. Introduction

Panax ginseng Meyer (Araliaceae), commonly known as Korean Ginseng, is one of the most widely used traditional medicines. *P. ginseng* roots are used as a tonic to enhance immune response and consequent health and longevity [1,2]. Diverse beneficial effects, such as anticancer, anti-diabetic, neuroprotective, and anti-inflammatory activities have also been reported [3–6].

To increase useful components and biological activities of Korean Ginseng, various preparation methods have been investigated. Drying after steaming, which produces Korean Red Ginseng (KRG), is well known for the production of new active constituents [7–10]. Fermentation or treatment in acidic conditions is also suggested for production of and/or increasing active constituents [11–14].

In order to use *P. ginseng* in traditional medicine or for development as functional foods, appropriate extraction procedures are indispensable. Extraction procedures are also important in determining extract efficacy. Many factors, such as extraction solvent, extraction time and temperature, and solid–liquid ratios, affect extract composition, as well as biological activity [15–17].

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Therefore, optimization of extraction conditions is required for maximum efficacy. Response surface methodology (RSM) is a useful statistical tool that can derive optimal conditions by considering several factors simultaneously. RSM consists of mathematical and statistical methods and derives optimal conditions based on experimental data obtained from rationally designed experiments [18–20]. Therefore, RSM is an effective method for optimization of extraction conditions, especially in cases involving multiple variables.

Oxidative stress describes an imbalance between the production of reactive oxygen species and antioxidant defenses. It is a major contributor to age-related symptoms and pathogenesis of many diseases, such as cancer, diabetes, atherosclerosis, neurodegenerative diseases, and osteoporosis [21,22]. Consumption of antioxidant-rich fruits or botanical extracts minimizes senescence and chronic disease [23–25]. KRG reportedly exhibits beneficial effects against various diseases through enhancing antioxidant defense [26–29].

In the present study, we investigated the impact of KRG extraction conditions on antioxidant activity using RSM. Given the importance of extraction efficiency for further product development, the extraction yield was also compared. Additionally, ginsenoside Rg1 and phenolic content were also measured. Ultimately, optimized extraction conditions for maximum antioxidant activity and maximum extraction yield using RSM are suggested.

2. Materials and methods

2.1. Plant material

KRG was purchased from a local herbal market in Chungbuk, Korea, in September 2014. They were identified by the herbarium of College of Pharmacy at Chungbuk National University, where a voucher specimen was deposited (CBNU201409-KRG). Ginsenoside Rg1 was purchased from Baoji Herbest Bio-Tech Co., Ltd (Baoji, Shaanxi, China).

2.2. Preparation of KRG extract

Powdered KRG (500 mg) was weighed and extracted with 10 mL extraction solvent as indicated in Table 1. The solvent was evaporated and the extract analyzed for antioxidant activity. For HPLC analysis, each sample solution was filtered through a 0.45 μm membrane filter.

2.3. Antioxidant activity

KRG antioxidant activity was evaluated by measuring free-radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, extracts prepared from different extraction conditions were mixed with freshly prepared DPPH solution. After shaking, the reaction mixtures were allowed to stand for 30 min at room temperature in a dark environment. The radical scavenging activity was determined by measuring the absorbance at 517 nm. The relative radical scavenging activity (%) was calculated as [1 − absorbance of solution with sample and DPPH / absorbance of solution with DPPH] × 100.

2.4. Experimental design for RSM

A Box-Behnken design (BBD) with three variables and three levels was used to optimize the extraction conditions of KRG. Target responses were selected as antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content. The independent extraction variables for extraction solvent (ethanol) (X_1), extraction time (X_2), and extraction temperature (X_3) were chosen for this study and their ranges determined based on a preliminary single-factor experiment. As shown in Table 1, the complete design consisted of 15 experimental points, including three replicates of the center points (all variables were coded as zero).

Regression analysis was performed according to the experimental data. The mathematical model is described by the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{1 \le i \le j}^{3} \beta_{ij} X_i X_j$$

where Y is the response, β_0 is the constant coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient. The statistical significance of the coefficients in the regression equation was checked by analysis of variance (ANOVA). The fitness of the polynomial model equation to the responses was evaluated with the coefficients of R^2 and lack of fit was evaluated using an F-test.

Table 1
A Box-Behnken design for independent variables and their responses

Run	Coded variables			Actual variables			Observed values			
	X_1	X_2	X_3	EtOH (%)	Time (min)	Temperature (°C)	Antioxidant activity (%)	Extraction yield (%)	Rg1 (mg/g extract)	Phenolics (mg GAE /g extract)
1	1	0	−1	100	60	30	7.9	1.6	23.0	2.8
2	0	0	0	50	60	60	40.9	19.7	3.3	7.4
3	0	−1	−1	50	30	30	36.2	18.2	3.7	7.3
4	0	0	0	50	60	60	40.3	20.1	3.3	8.7
5	0	1	1	50	90	90	35.6	27.9	2.2	10.2
6	1	−1	0	100	30	60	18.9	5.4	14.3	3.7
7	0	0	0	50	60	60	40.1	18.2	3.4	7.3
8	1	1	0	100	90	60	25.6	5.4	21.5	4.0
9	−1	0	1	0	60	90	20.2	27.0	1.3	7.8
10	0	−1	1	50	30	90	39.7	23.1	2.5	9.8
11	−1	0	−1	0	60	30	29.2	24.0	1.7	8.8
12	−1	1	0	0	90	60	25.0	21.3	1.8	6.6
13	−1	−1	0	0	30	60	20.2	24.5	2.0	8.3
14	0	1	−1	50	90	30	36.3	16.3	3.7	8.8
15	1	0	1	100	60	90	32.8	8.0	16.0	5.2

EtOH, ethanol.