OPTIMIZATION OF THE ANTIOXIDANT EXTRACTION FROM *ELEUTHEROCOCCUS SENTICOSUS* ROOTS BY RESPONSE SURFACE METHODOLOGY

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Abstract: *Eleutherococcus senticosus* is known as adaptogen with benefits in general health promotion. The aim of this study was to investigate the effect of major extraction parameters on extraction yield of antioxidants measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. Secondly, content of total polyphenols was evaluated. Optimal conditions of the extraction were processed by response surface methodology. The independent variables of extraction were proposed as temperature, solid–liquid ratio and solvent composition. For the optimal antioxidant extraction, *E. senticosus* is suitable to extract by 23 % (v/v) aqueous ethanol at 70 °C in ratio 53 mL of extraction solvent per g of plant material. The optimal conditions calculated for the extraction of total polyphenols were very similar (70 °C, 22 % (v/v) aqueous ethanol) expect solid-liquid ratio which indicates need of increasing of solid-liquid ratio to 91 mL of extraction solvent per g of plant material.

Key words: extraction, *Eleutherococcus senticosus*, antioxidant activity, polyphenols, response surface methodology

1. Introduction

Eleutherococcus senticosus, known as Siberian ginseng or *Acanthopanax senticosus*, belong to the *Araliaceae* family, is distributed in China, Korea and Russia. *E. senticosus* has been known in traditional Chinese medicine due to putative benefits in general health promotion, vitality, stamina, restoration of homeostasis, chemoprevention, wound healing, longevity and other indications (KITTS and HU, 2000). By detailed research, it was found that the active compounds of *E. senticosus* include ginsenosides, polysaccharides, peptides, polyacetylenes, phenols and enzymes (XIANG *et al.,* 2008). The major active compounds are acanthoside D, eleutherosides I, K, L, M (triterpenic saponins), eleutherosides B, B_1, D, E (phenylpropane derivates) (KITTS and HU, 2000), senticoside, β-sitosterol, sesamin (DAVYDOV and KRIKORIAN, 2000; LEE *et al*., 2004; LI *et al*., 2006). Today, the extracts from different parts of *E. senticosus* are studied for their biological and pharmacological

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effects include antioxidant, antidiabetes, anticancer, anti-inflammatory, imunoregulatory and immunomodulating, antimicrobial and antiviral activities (FUJIKAWA *et al*., 1996; HIBASAMI *et al*., 2000; YI *et al.*, 2002; JUNG *et al*., 2003).

Several works are focused on the medical efficacy of *E. senticosus* mediated via its antioxidant actions, e.g. inhibiting lipid peroxidation and oxidative injury to DNA (LEE *et al.*, 1998; NAVAL *et al.*, 2007; LEE *et al.*, 2008; CHEN *et al.*, 2010; LEE *et al.*, 2011). Except for protection against oxidative reaction, the antioxidant activity of *E. senticosus* is associated with their traditional using as adaptogen by the prevention of human body against faster organ aging.

Antioxidants from *E. senticosus* belonging to polar and less polar components can be extracted by various extraction solvent at different extraction conditions. Determination of optimal values of extraction parameter is significant point in design of extraction process for obtaining of biological active compounds from plant material. In this regard, the response surface methodology (RSM) is a powerful tool that can provide a complete optimal condition to improve a various technology processes (BOX and WILSON, 1951). It is a collection of statistical and mathematical techniques and it has important applications in the design, development and formulation of new products as well as in the improvement of existing products (MYERS and MONTGOMERY, 1995).

The aim of this study was to evaluate the effects of the extraction temperature, solvent composition and solid-liquid ratio on the extraction yield of antioxidants. Secondary, total polyphenol content was determined to find possible relations between this parameter and antioxidant activity. This work provides preliminary data for making products from *E. senticosus* with high antioxidant activity which would be used as food additives or nutraceutical supplement with specific effects to human health.

2. Material and methods

2.1 Chemicals

Ethanol 96 %, methanol, Folin-Ciocalteu reagent and sodium carbonate were obtained from Mikrochem (SK). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were obtained from Sigma-Aldrich (D).

2.2 Plant material

The roots of *E. senticosus* (F-Dental Hodonin, CZ) were an aliquot amount of the tested material cut at particle size < 0.1 cm.

2.3 Extraction procedure

For determination of optimization ranges, 1 g of dried roots of *E. senticosus* was extracted by extraction solvent (aqueous ethanol with concentration 0, 10, 30, 50, 70, 90 and 96 % (v/v) of ethanol) in solid-liquid ratio (10, 25, 50, 75 and 100 mL per gram of plant material) at various temperatures (20, 30, 50 and 70 °C) during 60 minutes. After 60 minutes, the solution was centrifuged for 2 minutes at 4,000 RPM and the supernatant was used for analysis.

2.4 Experimental design

Three factors, five level experiment was carried out with tested, independent variables- temperature (22, 34, 52, 70 and 82 °C), solvent composition (3.3, 10, 20, 30 and 36.7 % (v/v) ethanol) and solid-liquid ratio (8.2, 25, 50, 75 and 91.8 mL/g). Real variables values were transformed into non-dimensional coded form (Table 1).

Table 1. Independent variables in original and coded form and experimental results for response variables TEAC [mg/g plant material] and total polyphenols [mg/g plant material].

Stand. order	**Temperature [°C]**	**Solvent composition [% EtOH]**	**Solid-liquid ratio [mL/g]**	**TEAC [mg/g plant material]**	**Total polyphenols [mg/g plant material]**
1	34 (-1)	10 (-1)	25 (-1)	15.0	8.8
2	52 (0)	20 (0)	50 (0)	30.9	18.5
3	34 (-1)	30 (1)	75 (1)	20.5	15.9
4	70 (1)	30 (1)	25 (-1)	31.5	17.5
5	70 (1)	10 (-1)	75 (1)	26.1	21.8
6	70 (1)	10 (-1)	25 (-1)	18.9	13.0
7	34 (-1)	30 (1)	25 (-1)	14.7	9.5
8	70 (1)	30 (1)	75 (1)	27.4	21.5
9	52 (0)	20 (0)	50 (0)	30.9	18.0
10	34 (-1)	10 (-1)	75 (1)	24.4	10.7
11	52 (0)	3.3 (-1.682)	50 (0)	18.2	9.8
12	52 (0)	20 (0)	91.8 (1.682)	23.3	20.2
13	82 (1.682)	20 (0)	50 (0)	26.4	12.8
14	52 (0)	20 (0)	8.2 (-1.682)	10.1	6.2
15	22 (-1.682)	20 (0)	50 (0)	17.9	9.1
16	52 (0)	36.7 (1.682)	50 (0)	14.3	12.3
17	52 (0)	20 (0)	50 (0)	28.9	17.5

Measured dependent variables were Trolox equivalent antioxidant capacity (TEAC) in mg/g of plant material and total polyphenols in mg/g of plant material. Experimental data were fit by the polynomial regression of the second order (Eq. 1), and regression coefficients (b_i) were calculated.

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2} b_{ij} X_i X_j \quad (1)$$

where X_i are independent variables responsible for response Y and b_i are regression coefficients, describing relations of the measured properties to coded levels of the selected parameters (Table 1). For computer and statistical processing, the Statgraphics Plus 5.1 (Statpoint Technologies, USA) was applied.

2.5 Analysis of the response variables

2.5.1 Trolox equivalent antioxidant capacity

TEAC was determined using the method proposed by YEN and CHEN (1995) by with modification to microplate form. DPPH (0.012 %; w/v) was dissolved in pure methanol. The radical stock solution was prepared fresh daily. The DPPH solution (100 μL) was added to 25 μL extract. The mixture was shaken and allowed to stand at room temperature for 10 minutes. The decrease in absorbance was monitored at 540 nm. The results were corrected for dilution and expressed in mg Trolox per gram of plant material.

2.5.2 Total polyphenols

Total polyphenol content was measured using Folin–Ciocalteu colorimetric method (SINGLETON *et al.*, 1999). Plant extracts (20 μL) were mixed with 20 μL Folin–Ciocalteu reagent and incubated at room temperature for 5 minutes. Following the addition of 200 μL 20 % Na_2CO_3 (w/v) to the mixture, total polyphenols were measured at 690 nm. The results were expressed as gallic acid equivalents, mg per gram of plant material.

2.6 Statistical analyses

All experiments were realized in triplicate and statistical analysis was calculated by Statgraphics Plus 5.1.

3. Results and discussion

3.1 Selection of optimization ranges

The extraction efficiency of natural compounds from the plant material is dependent on some parameters such as temperature, time, solid-liquid ratio, solvent composition and others. All these parameters were put in optimization aimed to maximal antioxidant activity. The polyphenol content in plant extracts is often employed to content of antioxidant constituents (WU *et al.*, 2004). This dependence was evaluated also in our work. The influence of some above-mentioned parameters was partly reported in literature (CHEN *et al.*, 2010; LEE *et al.*, 2011; PARK *et al.*, 2006; WANG, 2012), but optimization for the extraction of antioxidants and polyphenols from *E. senticosus* using RSM have not been reported. In the literature, for antioxidant extraction, various extraction solvents such as water, methanol, acetone or their water solutions was used (PARK *et al.*, 2006; WANG, 2012). For this purpose, methanol appeared to be an effective extraction solvent.

The temperature plays an important role in the extraction of active substances from plant materials. In this work, we studied antioxidant extraction from *E. senticosus* at four different temperatures: 20, 30, 50 and 70 °C by measuring kinetics of extraction within 1 hour because it was necessary to determine the extraction time as a fixed parameter in the optimization. After one hour of extraction, the antioxidant activity of prepared extracts no longer increases (Fig. 1). Therefore, in all experiments, we used extraction parameter – time as fixed parameter with value - one hour. According to Fig. 1, the most suitable temperature for extraction of antioxidants from *E. senticosus* was 70 °C. In the literature, the extraction of antioxidants was carried out at 70 °C by distillated water during 3 hours (PARK *et al*., 2006). On the other hand, KIM *et al.* (2002) and LEE *et al.* (2011) have done extraction antioxidant activity at laboratory temperature. On the basis of this information, we have been selected the temperature range of 34 – 70 °C.

Transport of active compounds from plant material to extraction solvent is diffusion process affected by various factors, but primary attribute of extraction is the solubilization of extracted compounds. Solubilization of target compounds is affected also by solubilization of ballast compounds. Therefore, selection of extraction solvent volume needed for extraction target compounds is very important. The highest antioxidant activity was measured at up to 50 mL/g of plant material. In the literature, authors used various solid-liquid ratios as 13.3 (LEE *et al*., 2011), 35 (WANG, 2012) and 100 mL/g of plant material (PARK *et al*., 2006). Therefore, it can be considered that optimal value of parameter solid-liquid ratio will be located between values 25 – 75 mL/g of plant material.

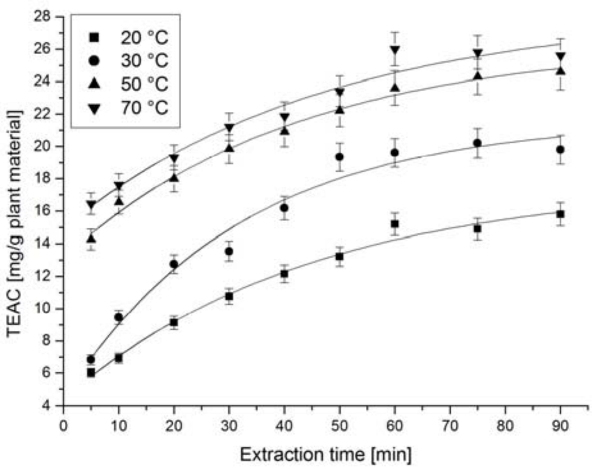

Fig. 1. The kinetic of antioxidant extraction by various temperature (20, 30, 50 and 70 °C) during 1 hour by 50 mL to g of plant material expressed as mg of TEAC per g of plant material.

In the context with solubilization of target compounds, solvent polarity is also important. For extraction of active compounds from *E. senticosus*, authors of other studies recommend using of polar extraction solvent such as ethanol or methanol and their water mixtures. Because methanol is toxic and unauthorized for food industry, aqueous solution of ethanol with concentrations of ethanol varied between 0 and 96 % (v/v) was used for antioxidant extraction from *E. senticosus*. The highest antioxidant activity was determined in extracts prepared by 10 and 20 % (v/v) aqueous ethanol. Opposed to the work of LEE *et al*. (2011) was the highest antioxidant activity in extracts prepared from *E. senticosus* by 40 – 80 % (v/v) aqueous ethanol. Based on the measured results, the optimization ranges of solvent composition were selected 10 – 30 % (v/v) aqueous solution of ethanol.

3.2 Extraction optimization by response surface methodology (RSM)

Optimal conditions of the extraction were calculated by the software Statgraphics Plus 5.1., processed by RSM approach. In Table 1, TEAC and polyphenols are presented. Based upon the regression analysis results, we can state, that compared dependent variable expressed self-independent relation.

3.2.1 Multiple linear regression

For the purpose of the fitting the presented results in Table 1, polynomial regression of the second order (Eq. 1) had regression coefficient $R^2 = 0.95$ for TEAC as parameter Y1, $R^2 = 0.91$ for total polyphenols as parameter Y2.

3.2.2 Regression coefficient analysis

Regression coefficients of the model for TEAC and total polyphenols obtained by multiple polynomial regression are presented in Table 2. Dependent variable in coded form (Table 1) allow direct interpretation of the effect (linear, quadratic and interaction) of the independent variables to dependent variables and visualization by 3D surface plots (Fig. 2) assisted visualization of the statistically important factors (marked as bold in the Table 2) obtained from statistical analysis.

Table 2. Regression coefficients of the model polynomial regression of the second order for dependent variables - TEAC [mg/g plant material] and total polyphenols [mg/g plant material].

Model parameters		**TEAC**	**Total polyphenols**
Constant effect		-34.11	-20.8457
Linear Effect	Temperature [°C] (A)	**0.673912**	**0.653699**
	Solvent composition [% EtOH] (B)	1.30007	0.83962
	Solid-liquid ratio [mL/g] (C)	**1.03881**	**0.213897**
Quadratic effect	A × A	-0.00558012	-0.0052778
	B × B	**-0.0390238**	-0.0166475
	C × C	**-0.00598639**	-0.00143291
Interaction effect	A × B	0.0125748	-0.00116015
	A × C	-0.00335388	0.00123142
	B × C	-0.00736462	-0.000160227

3.3 Determination and experimental validation of the optimal conditions

Optimal values of the parameters for extraction TEAC and total polyphenols from *E. senticosus* are presented in Table 3. Predicted value for extraction TEAC (70 °C, 53 mL per g of plant material, 23 % (v/v) aqueous ethanol) and total polyphenols (70 °C, 91 mL per g of plant material, 22 % (v/v) aqueous ethanol) from *E. senticosus* as dependent parameters were comparable with experimentally measured value at the level of the statistical significance at $p < 0.05$. Achieved results confirm the possibility to predict the course of the extraction of active substances from *E. senticosus* by the model under particular experimental conditions.

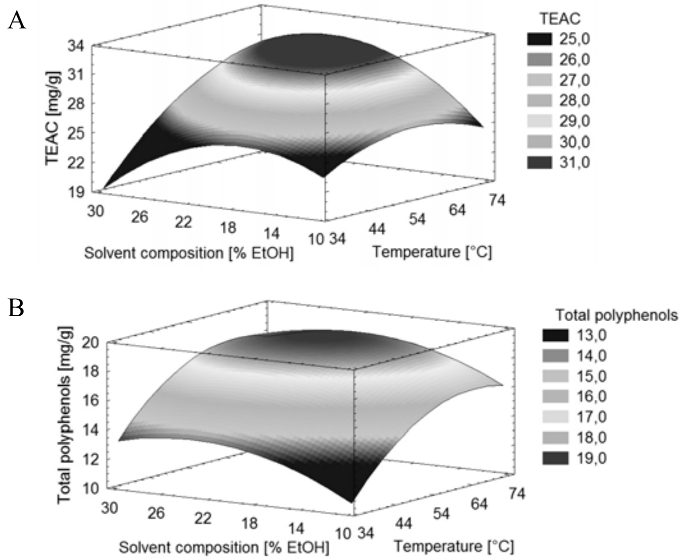

Fig. 2. The relation of the dependent variables from the temperature and solvent composition at constant solid-liquid ratio 50 mL per g of plant material; A – TEAC [mg of TEAC per g of plant material]; B – total polyphenols [mg of gallic acid per g of plant material].

Table 3. Optimal extraction parameters for maximizing yield of TEAC and total polyphenols and comparison of the predicted values of the dependent variables and experimentally measured values at these optimal conditions.

Optimal extraction parameters		
Temperature [°C]	70	70
Solvent composition [% EtOH]	23	22
Solid-liquid ratio [mL/g]	53	91
	TEAC [mg/g plant material]	Total polyphenols [mg/g plant material]
Predicted values	32.1	22.8
Experimental values	31.4	21.3

In the work Lee et al. (2011) not found the optimal conditions for antioxidant extraction from *E. senticosus*. They describe that both concentration ethanol in extraction solvent and extraction time weren´t affected antioxidant extraction. In comparison with our experiment, they used low value of solid-liquid ratio, which cannot allow achieving sufficient antioxidant extraction.

Conclusions

The response surface methodology was successfully employed to optimize the antioxidant extraction from *E. senticosus*. The second-order polynomial model gave a satisfactory description of the experimental data. The optimal conditions for antioxidant extraction were 70 °C, 53 mL per g of plant material, 23 % (v/v) aqueous ethanol and the optimal conditions for the extraction of total polyphenols were 70 °C, 91 mL per g of plant material and 22 % (v/v) aqueous ethanol. Predicted values of dependent variables were comparable with the experimentally measured values.

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