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OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *SALVIA VERTICILLATA* L. ROOT USING RESPONSE SURFACE METHODOLOGY

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Abstract: This study aimed to optimize the conditions of ultrasonic-assisted extraction to obtain the highest content of phenolic compounds from *Salvia verticillata* L. root using response surface methodology. RSM was applied to maximize total phenolic content from *Salvia verticillata* L. root while minimizing operative temperature, ethanol composition, the solvent-to-solid ratio, and time. The obtained optimal conditions were ethanol percentage of 20 %, solvent-to-solid ratio of 40 mL g^{-1}, a temperature of 33.68 °C, and a time of extraction of 10 minutes. Obtained optimal conditions could be used for further investigation of the biological activities of *Salvia verticillata* L. and related plant species.

Keywords: *Salvia verticillata* L., ultrasonic extraction, computer optimization, total phenolic content, response surface methodology

Introduction

The extraction procedure is one of the most essential steps in the determination, separation, and identification of compounds in analyzed plant materials. The use of ultrasound for phenolic compound extraction has been reported as a faster, solvent-saving, and more efficient technique than traditional extraction procedures (Tao et al., 2014). A cavitation effect generated by ultrasonic waves may accelerate the release of target compounds and speed up the extraction process. Several factors, including temperature of extraction, used solvent composition, extraction time, and solvent-to-solid ratio may impact the ultrasonic-assisted extraction (UAE) efficiency (Zhou et al., 2017).

Salvia verticillata L. is (lilac sage) belongs to the genus *Salvia* (Lamiaceae). Salvia plants have applications in the food, aromatic, and pharmaceutical

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industries. *Salvia verticillata* L. is traditionally used as an expectorant, for disinfection of the oral cavity, and wound healing (Jarić et al., 2015). *S. verticillata* contains polyphenols and diterpenoids and is recognized for its antioxidant properties. According to certain studies, *S. verticillata* is a significant source of rosmarinic and salvianolic acid and its derivatives (Šulniūtė et al., 2017). Several papers have mentioned the plant's biological activity, including its antibacterial and anti-diabetic properties, but also the potential of being used in lost or declining cognitive functions (Naderi et al., 2011).

Considering the potential of this plant and bearing in mind that the *S. verticillata* root research is negligible compared to the aerial part, the idea of this study was to find the optimal conditions for ultrasonic-assisted extraction of *S. verticillata* root, in order to maximaze the content of total phenolic compounds, while utilizing a minimal amount of solvent and short extraction time, all while maintaining the lowest possible extraction temperature. The obtained results can be applied to additional evaluations of antimicrobial activity, antioxidant activity, phenolic composition, etc.

Materials and methods

Chemicals and plant material. Folin-Ciocalteu reagent used for the determination of total phenolic content was purchased from Sigma Aldrich (Deisenhofen, Germany). Root parts of *S. verticillata* were collected in October 2017, at the locality near Kragujevac, Central Serbia. The identification of species was carried out at the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac (Kragujevac, Serbia). After being cleaned of impurities, plant portions were placed in a dark, well-ventilated room to dry. The dry root parts were finely powdered and 1 g of each was measured.

Software. The experimental design, data analysis, and desirability function calculations were performed using Design Expert trial version 13.0 (Stat-Ease Inc., Minneapolis, MN, US).

Preparation of the *S. verticillata* extracts and experimental design for extraction optimization. Samples (1 g) were extracted using ethanol : water solvent with varying ethanol composition (20, 60, 100, v/v%) at different temperatures (25, 50, 75 °C) and for different extraction times (10, 20, 30 min) in an ultrasonic bath (Bandelin Sonorex RK 52 H, Bandelin electronic GmbH & Co. KG, Berlin, Germany) which was coupled with circular thermostat (Lauda Alpha A6, LAUDA-Brinkmann, Delran, NJ, USA) to keep the temperature constant and prevent overheating.

Spectrophotometric determination of phenolic compounds. The Folin-Ciocalteu procedure was applied to determine the total phenolic content in *S. verticillata* extracts (Singleton et al., 1999). Plant extracts were diluted with methanol to a concentration of 0.4 mg mL^{-1}, and 1.25 mL of Folin-Ciocalteu reagent (previously diluted ten-fold in water) was mixed with 1 mL of 7.5% aqueous NaHCO3 solution and added in 0.25 mL of extracts. This reaction mixture was incubated at 45°C for 15 min and the absorbance was measured at 765 nm. Known concentrations of gallic acid were used to obtain a standard curve, and the results were expressed in milligrams of gallic acid equivalents (mg GAE g^{-1} dry weight of extracts).

Results and discussion

Four extraction variables (solvent volume, ethanol composition, sonication time, and extraction temperature) were optimized by statistical experimental design to produce the best yields of total phenolics. The recovery of phenolics is influenced by the temperature, duration, and solvent-to-solid ratio during extraction, resulting in inconsistent outcomes regarding the solubilization and breakdown of phytochemicals (Robards, 2003).

Optimization of the extraction procedure was performed by response surface methodology (RSM) with central composite design (CCD). The CCD experimental design is given in Table 1. Solvent-to-solid (range 10-40 mL g^{-1}), ethanol concentration (20-100%), time (10-30 min), and temperature (25-75 °C), and central points were selected based on the initial experimental results and literature data. The independent variables are coded at three levels (–1, 0, and +1) and the experimental design consisted of 30 experimental points.

The selection of the best conditions for the extract preparation was carried out using multi-response analysis following the methodology presented by Derringer & Suich (Derringer and Suich, 1980).

The response in the experimental design was calculated as the total amount of phenolics, and its range was 5.41 – 58.2 mg GAE g^{-1} dry weight of *S. verticillata* root. Taking into consideration the range of input variables, and in order to obtain the maximum quantity of phenolic compounds from the extract, the optimal conditions were determined - ethanol percentage of 20 %, solvent-to-solid ratio 40 mL g^{-1}, temperature of 33.68 °C and extraction time of 10 minutes, with desirability of 0.862. Total phenolic content obtained under optimal conditions was 40.79 mg GAE g^{-1} DW of *S. verticillata* root.

Table 1. Central composite design (CCD) with the responses of the dependent variables

Run	EtOH (%)	Solvent-to-solid (mL g^{-1})	Time (min)	Temperature (°C)	Total phenolics (mg GAE g^{-1} DW)
7	20	10	10	25	5.91951
24	100	10	10	25	12.1549
23	20	40	10	25	34.2679
4	100	40	10	25	21.3958
16	20	10	30	25	5.41009
14	100	10	30	25	14.4381
18	20	40	30	25	39.9857
27	100	40	30	25	29.9541
30	20	10	10	75	14.9516
20	100	10	10	75	14.6409
8	20	40	10	75	46.4029
26	100	40	10	75	30.6877
22	20	10	30	75	6.04177
13	100	10	30	75	9.93989
10	20	40	30	75	58.3225
25	100	40	30	75	46.2557
1	20	25	20	50	31.1004
6	100	25	20	50	26.2608
3	60	10	20	50	33.1223
12	60	40	20	50	56.3627
17	60	25	10	50	28.5023
19	60	25	30	50	29.5721
15	60	25	20	25	22.4834
29	60	25	20	75	25.512
11	60	25	20	50	32.4962
5	60	25	20	50	29.1136
28	60	25	20	50	30.54
9	60	25	20	50	31.2532
2	60	25	20	50	33.189
21	60	25	20	50	32.5003

The response surface for extraction of total phenolic compounds is given in Figure 1, showing the most significant interaction between input extraction variables. The significance of the effects of the independent variables and their influence on the dependent variables was checked using ANOVA analysis.

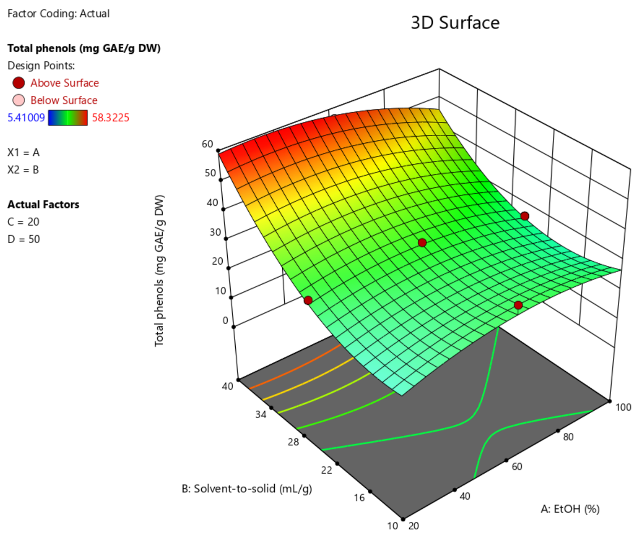

Figure 1. Response surface for extraction of total phenolic compounds; significant interaction between extraction variables

The variables with the highest influence on the extraction efficiency were solvent-to-solid ratio and temperature of extraction (highly significant, $p < 0.0001$), but also interaction between extraction variables solid-to-solvent ratio and ethanol composition ($p < 0.0001$). The model F-value of 53.42 implies the model is significant. The predicted R^2 of 0.8832 is in reasonable agreement with the adjusted R^2 of 0.9620; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio and obtained ratio of 28.181 indicates an adequate signal, along with a coefficient of variation of 9.57%.

Conclusion

In this work, the conditions of ultrasonic-assisted extraction of phenolic compounds from the *Salvia verticillata* L. root were simultaneously optimized using statistical tools - central composite design and Deringer's function, which enable detail insight into the extraction process. The proposed method for total phenolics extraction is fast, simple, low-temperature and does not involve large amounts of harmful chemicals. Obtained optimal conditions could be applied for further investigation of the biological activities of *Salvia verticillata* L. and related plant species.

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