JRIFST

www.journals.rifst.ac.ir
Journal of Research and Innovation
in Food Science and Technology

Research Institute of Food Science and Technology

Volume 9, Issue 2, Summer 2020, Pages 113-126
Document Type: Extended Abstract
DOI: 10.22101/JRIFST.2019.11.10.e1085

Extended Abstract

The Optimization of Solvent Extraction Process from *Salvia Leriifolia* Leaf Extract Containing Antimicrobial Compounds Using Response Surface Methodology (RSM)

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Received: 2019.05.13; **Accepted**: 2019.11.09

Abstract

In this research, determining the optimal conditions for extraction of *Salvia leriifolia* leaf by traditional solvent extraction method (with different solvent ratio of water/ethanol 50:50, 60:40 and 70:30 at temperatures of 70, 80 and 90 ºC and time duration of 30, 75 and 120 min) in order to investigate the antimicrobial activity of the extract, using response surface method was carried out. The analysis of variances showed that the effect of all three independent variables on the dependent variables (the diameter of the inhibition zone of *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Salmonella enteritidis, Saccharomyces cerevisiae* and *Aspergillus niger*) was significant (P<0.05). Optimum extraction conditions including temperature of 83.60 ºC, extraction time of 60.92 min and water to ethanol ratio of 59.09 were obtained. Regarding the diameter of the inhibition zone of microorganisms, the highest inhibition zone belonged to *Saccharomyces cerevisiae* and *Staphylococcus aureus*.The optimization of extraction method resulted in extracts with the highest antimicrobial activity, especially against yeast and Gram-positive bacteria. In addition, predicting evaluation models provided an effective step in selecting extraction conditions to achieve a combination of antimicrobial properties against each microbial group.

Keywords: Antimicrobial compounds, Response surface methodology, *Salvia leriifolia*, Traditional solvent extraction

Introduction

Salvia leriifolia, known as Nowroozak, belongs to the genus Salvia (Lamiaceae) (Rechinger, 1982). This plant has 56 species in different parts of Iran (Ghahreman, 1994). The studies indicate considerable antimicrobial activity in different parts of this plant such as root and leaf (Baghi, 1996; Jabbarzadeh, 1999; Modarres & Abrishamchi, 2008, 2010). The high antimicrobial activity in the leaves is due to the presence of valuable secondary metabolites such as terpenoids, saponins, flavonoids, tannins and alkaloids (Tabatabai Yazdi, 1995).

Phenolic acids including caffeic acid, rosmarinic acid, and salvianolic acid B have been identified and quantified in leaf and root of Nowroozak by HPLC and rosmarinic acid content was predominant (Modarres *et al*., 2014). A previous study has demonstrated that the antimicrobial activity of extract and essential oil of *Salvia leriifolia* on different microorganisms (*Staphylococcus aureus*, *Bacillus Subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*) (Baghi, 1996). Also in another study, the effect of Nowroozak leaf extract powder against total microbial count and *staphylococcus aureus* in hamburgers during 45 days storage time was significant (Yousefli, Hosseini, Haddad Khodaparast, Azarnivand, & Pezeshki, 2011).

Although there are various methods for extracting, the traditional solvent extraction is general method (Adámez, Samino, Sánchez, & González-Gómez, 2012). In this method sample being exposed to a liquid solvent. Crushing the sample and soaking it in the solvent is a common method that is performed prior to extraction to reduce the particle size of the sample to perform a better diffusion mechanism (Chan, Yusoff, & Ngoh, 2014).

Response surface methodology (RSM) is a statistical method that uses quantitative data from the related experiment to determine regression model and to optimize a response which is influenced by several independent variables. This method is used to describe the individual and interaction effects of independent variables on responses.

The aim of the present study was to optimize the extraction conditions of extracts containing antimicrobial compounds from Nowroozak leaf using response surface methodology to obtain the most antimicrobial activity against some of food pathogen microorganisms.

Materials and methods

In order to obtain the maximum efficiency and antimicrobial properties of Nowroozak extract, the extraction was performed at different temperatures (70, 80 and 90 °C), different times (30, 75, and 120 min) and different ratios of water to ethanol (50:50, 60:40, and 70:30). The ratio of solid to solvent was 1:10 w/v. The extracts were centrifuged for 10 min and the supernatants were collected and dried at 40 °C (Hayrapetyan, Hazeleger, & Beumer, 2012).

Duo to investigation of the inhibition zone diameter of Nowroozak leaf extract on *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella enteritidis, Saccharomyces cerevisiae* and *Aspergillus niger*, 100 μL of suspension of microorganismes were spread on Muller Hinton Agar (for bacteria) and Yeast and Mold Agar (for mold and yeast). Also 100 μL of extract with a concentration of 500 mg/mL was poured into the wells. The plates were incubated at suitable temperature for certain time and then the diameter of the inhibition zone around the wells was measured (Iturriaga, Olabarrieta, & de Marañón, 2012).

In this study, statistical analysis carried out by response surface method based on central composite design with 3 replications at central point using Design Expert software version 7 (Stat -Ease Inc., Minneapolis, Minn., USA).

Results and discussion

Statistically, the model proposed by the software to predict the effect of independent variables, interactions, and quadratic effects on the diameter of inhibition zone of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enteritidis* was polynomial.

The highest and lowest inhibition zone diameter of *Listeria monocytogenes*, were 22 and 8 mm respectively. Also the results of analysis of variance showed that significant expressions of the model included linear effect of extraction time and quadratic effect of water/ethanol solvent ratio. The diameter of inhibition zone of *Staphylococcus aureus* was calculated between 10 and 23 mm. Results of analysis of variance showed that the significant

expressions of the model included the interaction between temperature and extraction time and the quadratic effects of temperature and water/ethanol solvent ratio.

Regarding the diameter of *Escherichia coli* inhibition zone, the interaction effects of temperature and time as well as the time and water to ethanol solvent ratio and quadratic effects of all three independent variables were significant (P<0.05). However, in relation to the diameter of inhibition zone of *Salmonella enteritidis*, the simple effect of time and quadratic effects of temperature and water-to-ethanol ratio were significant (P<0.05). With increasing temperature from 70 to 80 °C, the inhibition zone of *Escherichia coli* increased, while higher extraction temperature caused a decrease in the diameter of inhibition zone. The highest diameter of inhibition zone of *Salmonella enteritidis* was 20 mm at extraction temperature of 80 °C, water to ethanol solvent ratio of 60:40 and time of 30 min.

Linear model as a suitable model has determined the diameter of inhibition zone of *Saccharomyces cerevisiae*; because only the simple effects of temperature and water-ethanol solvent ratio are significant. With respect to the diameter inhibition zone of *Aspergillus niger*, the predicted model is a quadratic multivariate model. In this regard, the simple and quadratic effect of temperature and the simple effect of water to ethanol solvent ratio were significant (P<0.05). In relation to *Saccharomyces cerevisiae*, with increasing extraction temperature from 70 to 90 °C, the inhibition zone diameter increased. The highest level was obtained at 90 °C with 27 mL. However, the extraction time and water-to-ethanol solvent ratio showed an inverse relationship with the inhibition zone diameter of this microorganism. As the temperature increases, the diameter of inhibition zone of *Aspergillus niger* decreased. Regarding the water-to-ethanol solvent ratio, the maximum diameter of the inhibition zone of *Aspergillus niger* was achived when the amount of water in the solvent system was 70%.

The optimization results of extraction from Nowroozak leaf extract by traditional solvent method with maximum values for all dependent variables were investigated simultaneously. The optimum temperature, extraction time and the ratio of water to ethanol solvent were 83.60 °C, 60.92 min and 59.09/40.91 respectively. After determining the optimum treatment, extraction was performed again and 19, 18, 15, 16, 19 and 11 mm diameter of inhibition zone for *Staphylococcus aureus*, *Listeria monocytogenes, Escherichia coli*, *Salmonella enteritidis*, *Saccharomyces cerevisiae* and *Aspergillus niger* were achieved respectively (Fig. 1).

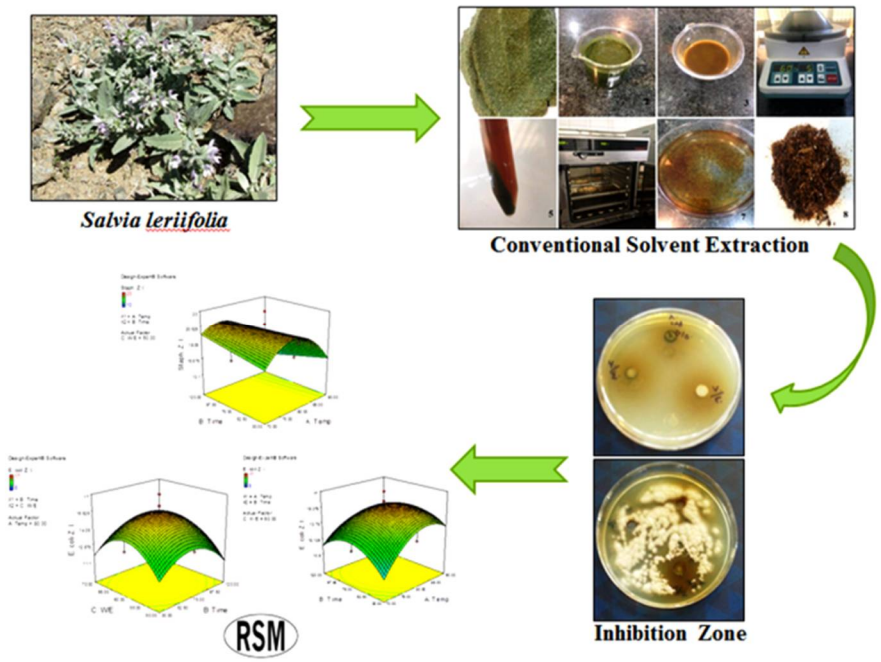

Fig. 1. Graphical abstract of the project

Conclusions

In the study, extraction of extract from Nowroozak leaf was performed using conventional solvent extraction method based on RSM and the antimicrobial potency of extracts on several microorganisms in foodstuffs including *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella cystis*, *Saccharomyces cerevisiae* and *Aspergillus niger* were investigated by measuring the inhibition zone diameter using the well diffusion method in order to predict the response behavior and the effects of simple, quadratic or interaction independent variables of extraction (temperature, time and water-ethanol solvent ratio), for each of the dependent variables. The results showed that the highest inhibition zone diameter belonged to *Saccharomyces cerevisiae* and *Staphylococcus aureus*. The optimization of extraction conditions and the use of predictive models resulted in the extract with the most antimicrobial properties against some of the most important food index microorganisms. Since the extraction method is effective on the type of components that is extracted, it is recommended to study the new extraction methods and optimization condition of each method to obtain the highest extraction of bioactive compounds, including those with antimicrobial properties.

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