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COMPARATIVE STUDY ON THE OPTIMIZATION OF MEDICINAL AND AROMATIC PLANTS EXTRACTION METHODS

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Abstract
Medicinal and aromatic plants (MAP) have always been an important element in maintaining and improving human health and vitality. The study includes medicinal and aromatic plants such as Satureja herba which have been selected for its high content of total phenols and antioxidant activity. Improvement of extraction methods is the main concern of the researchers for an efficient material exploitation and reduction of quantities of plant waste resulted from the extraction processes. Therefore, we have opted in this study for a comparison between three MAP extraction methods using modern methods. The optimization study consists in the usage of experimental design statistical techniques based on polynomial equations; the validation of these proposed optimization models is performed by evaluating the multiple regression coefficient, by performing the statistical analysis of the variance (ANOVA) or by plotting the response surfaces. In this study, favorable conclusions can be drawn regarding the selection of the optimal extraction parameters for each method, following an optimal ratio between the extraction results and material resources used in the process.

Keywords: medicinal and aromatic plants (MAP), optimization extraction methods, Satureja herba.

1. INTRODUCTION

Medicinal and aromatic plants (MAP) play an important role in health and are used in many areas, both as a laboratory study and as finished products. *Satureja herba* has numerous advantages. This plant is used in pharmacology as antiseptic, eupeptic; has antimicrobial, antioxidant, antiinflammatory, antispasmodic, antinociceptive and analgesic properties (Nascimento et al., 2000; Hajhashemi et al., 2002; Bozin et al., 2006; Jafari et al., 2016).

Obtaining extracts from plant material requires the use of some technologies and well-developed extraction techniques, to obtain extracts with high content of active principles and compounds.

The optimization of the extraction methods of the compounds of interest (especially the total phenols content, but also the antioxidant activity) from *Satureja herba* is the subject of this study.

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2. MATERIALS AND METHODS

Satureja herba was the medicinal and aromatic raw plant material used for the present work. Regarding the study of the optimization of the extraction methods of the selected plant, three different extraction techniques and methods were used, following the total phenols content and antioxidant activity for this plant.

The methods used are: microwave assisted by gravity hydrodiffusion (Microwave Extractor - Milestone ETHOS X Equipment), solvent-free microwave extraction (Microwave Extractor - Milestone ETHOS X Equipment) and accelerated solvent extraction (Dionex ASE 350 Equipment).

In order to optimize the extraction method of the active principles of MAP plants have been used the experimental design adaptable to each method. Was followed he influence of independent variables on the global extraction process of the active compounds of the vegetal product, selecting as dependent variables (responses) the total content of polyphenols and the antioxidant activity.

The relationship between independent and dependent variables is expressed by a polynomial equation of a certain degree, depending on the chosen model and the evolution of the responses in the experimental field, as:

$$Y = b_0 + \sum_{i=0}^{n} b_i X_i + \sum_{i<j}^{n} b_{ij} X_i X_j + \varepsilon$$

where: Y represents the measured response; b_0 represents the mean value of the Y response; b_i represents the main effects of the independent variables X_i; b_{ij} are the terms that give the measure of the interaction between the variables and ε is the random error.

Factorial analysis and experimental plans help to identify and explain the effects of several factors simultaneously, as well as to analyze their relative importance for the intended responses.

Evaluating the multiple regression coefficient, analysis of variance (ANOVA) and tracing the response surfaces are the statistical means used to validate the proposed model.

3. RESULTS AND DISCUSSIONS

Microwave assisted by gravity hydrodiffusion extraction method consists in obtaining plant extracts using microwaves. To optimize the method of extraction of pharmacologically active compounds from *Satureja hortensis* was used an experimental factorial plan 2x2. The influence of two independent variables, such as *the humidity* of the vegetal product undergoing the extraction process and *the microwave power* on the global process of extraction of the active compounds from the vegetal product were studied, and was selected as dependent variables (responses) the total polyphenol content, as well as the antioxidant activity of the resulting extract.

Humidity ranging from 25% to 50% and microwave power from 250W to 500W were been varied.

After analyzing the influence of independent variables on the concentration of *phenolic compounds* (expressed in Gallic acid) from the resulted extracts, was obtained the adjusted coefficient of determination (Adjusted R-squared) value as 0.9887. The adjustment of the R-squared (R^2) values according to the degree of freedom of the experimental plane is marked with Adjusted R-squared.

The analysis of variance (ANOVA) indicates a positive influence of humidity of the plant material in phenols compounds extraction (p=0.0424), while the microwave power had no statistically significant influence.

Figure 1 shows the combined effects and interactions between the selected independent variables on the extraction process of phenolic compounds from *Satureja hortensis*.

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After analyzing the influence of independent variables in *antioxidant activity* from the resulted extracts, was obtained the adjusted coefficient of determination (Adjusted R-squared) value as 0.9940.

ANOVA test shows a good fit for humidity influence (p=0.0294) and no statistically significant influence for microwave power, similar to phenols content case.

Figure 2 shows the combined effects and interactions between the independent variables selected on the antioxidant activity of *Satureja hortensis* extracts.

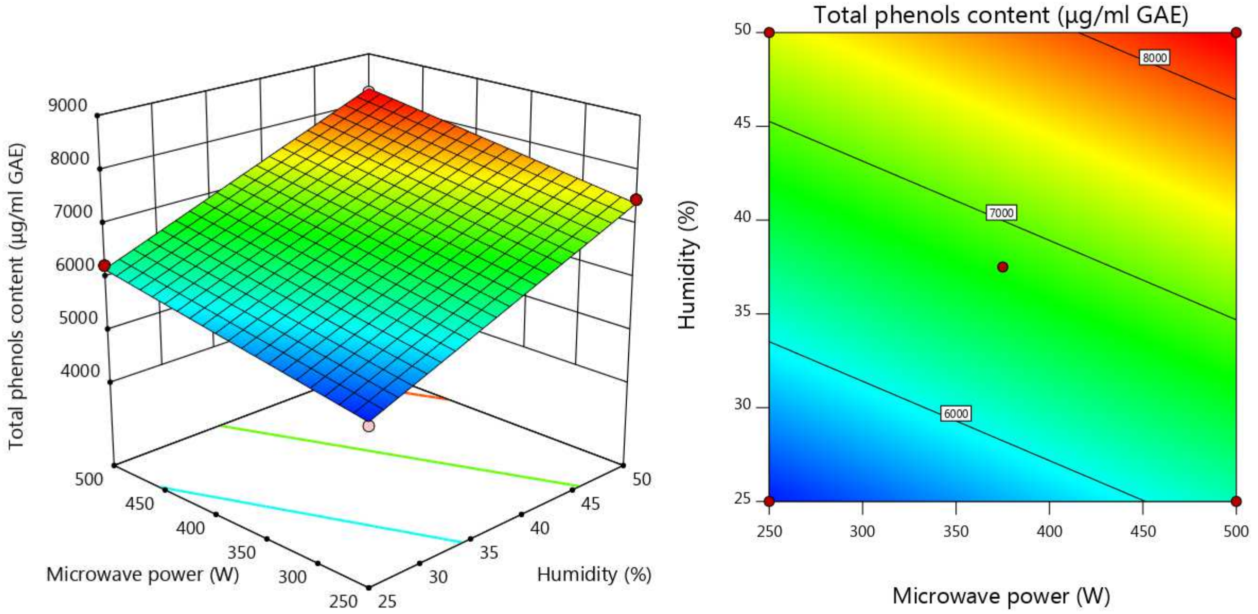

Figure 1. Response surfaces for the total phenolic concentration according to the extraction process variables, in 3D representation and two-dimensional projection respectively.

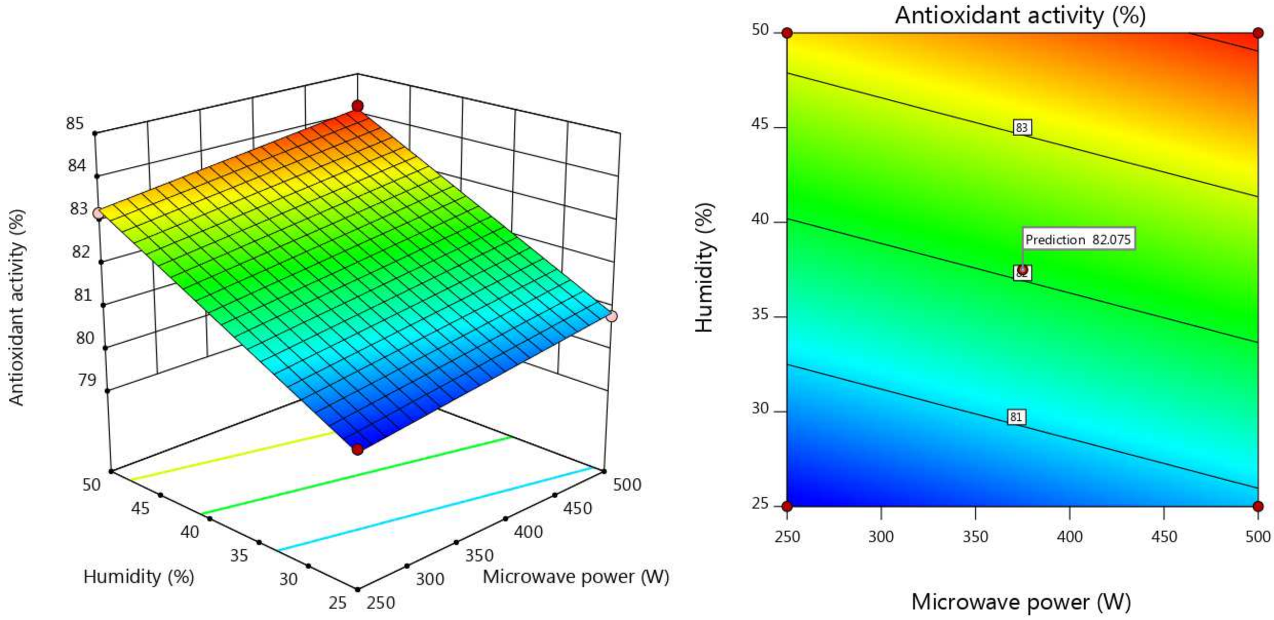

Figure 2. Response surfaces for antioxidant activity depending on the extraction process variables, in 3D representation and two-dimensional projection respectively

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Were imposed the folowing conditions to optimize the pharmacologically active compounds extraction, using this method, such as: the total phenols content and antioxidant activity to be maximal. In this case, the optimal parameters were 50% for humidity and microwave power 500W, to obtain 8305,5 μg/ml GAE total phenols content and 84.125% antioxidant activity.

Accelerated solvent extraction is an extraction method which uses ordinary solvents at high temperatures and pressures to achieve greater efficiency in the extraction process. The experimental factorial plan proposed for this method is 2x2, and the independent variables are solvent concentration (Ethanol) and the purge time. This study followed the influence of this numerical variables in the biological active compounds extraction as total phenols content (GAE μg/ml) and the antioxidant activity.

The Ethanol solvent concentration varied between 50%-100% and the purge time between 100-120 seconds.

Considering *the total phenols content*, was obtained the adjusted R-squared value as 0.9999. But, from ANOVA test is distinguish that the solvent concentration has a signifiant influence (p=0.0040) than the purge time variable, which denotes that the proposed model is statistically significant.

The effects of the selected independent variables in total phenols content study are represented in figure 3.

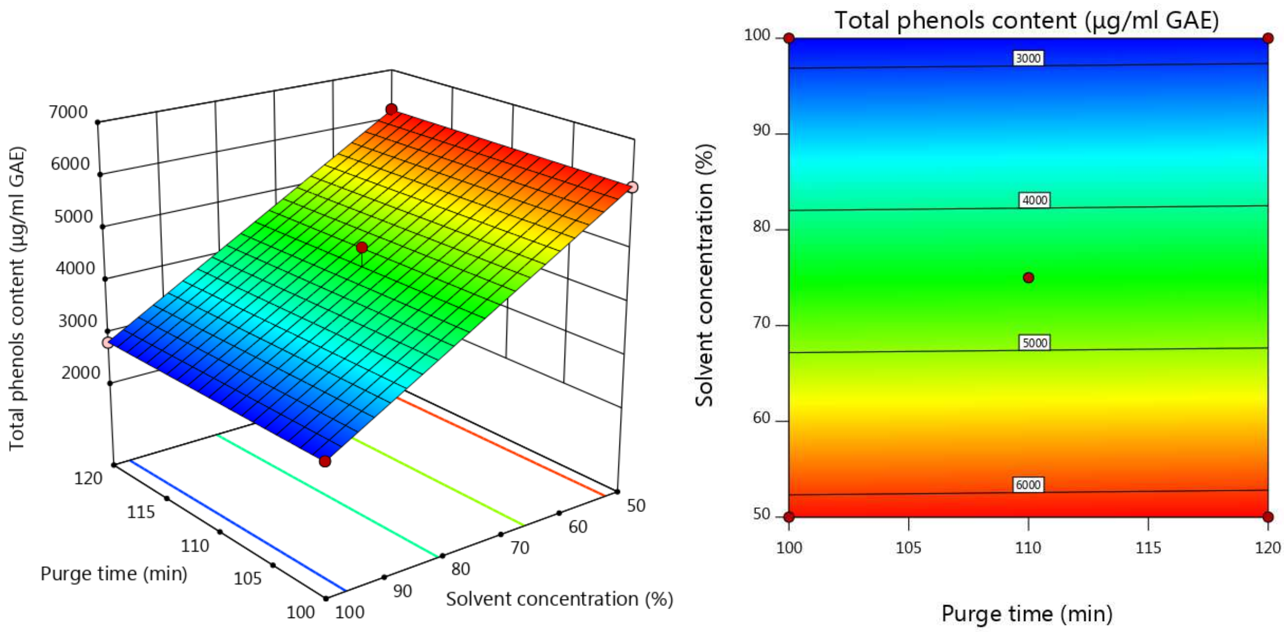

Figure 3. Response surfaces for the total phenol concentration (3D - left and 2D - right)

Antioxidant activity study leads to getting the adjusted coefficient of determination value as 0.9144. Instead, the ANOVA test indicates a weaker influence of the two independent variables selected in the model than in the study of the total phenols content. The effect of the variables selected in the antioxidant activity study can be observed in figure 4.

In order to optimize the extraction of pharmacologically active compounds from *Satureja hortensis* using the accelerated solvent extraction method, the following conditions were imposed as total phenols content to be maximum and the antioxidant activity to be at least 82%. This latter condition was posed because, surprisingly, the concentration of the solvent had an antagonistic effect on the two observed responses: it had a severe negative effect on the phenolic content of the extracts, but

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had a weak positive effect on their antioxidant activity. Therefore, it is not possible to identify experimental conditions that maximize both responses. In this situation, we adopted the optimal values of parameters as ethanol concentration 54.35% and the purge time 120 seconds to obtain 5896.72 µg/ml GAE total phenols content and 82% antioxidant activity.

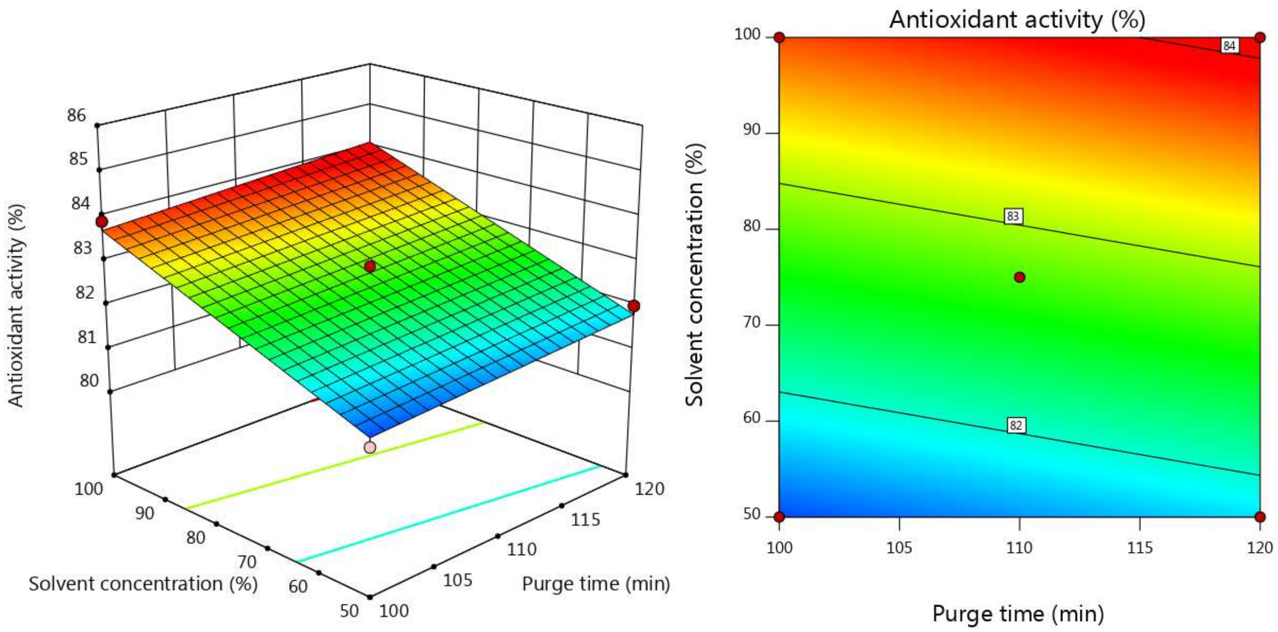

Figure 4. Response surfaces for the antioxidant activity in accelerated solvent extraction method (3D - left and 2D - right)

Solvent-free microwave extraction is an extraction method based on microwave and without the usage of the extraction solvents. In this method was adopted an experimental factorial plan 2x3, with microwave power, extraction time and extraction temperature as independent variables. The analysed responses were the total phenols content (µg/ml GAE) and antioxidant activity (%).
Microwave power ranged from 400-500W, the extraction time ranged from 20-30 minutes and the temperature from 75-95 ^{0}C.
In *the total phenols content* determination regarding this model, was obtained the 0.9464 value for the adjusted coefficient of determination value. ANOVA test indicates a positive and significant influence of each selected factor (microwave power p=0.0025; time p=0.0154 and temperature p=0.0463) and a certitude that the model is statistically significant for this method.
The mixed effects of these three variables are presented in figures 5, 6 and 7.
The work about the *antioxidant activity* developed in this method, showed an adjusted coefficient of determination value as 0.9500. From ANOVA test we can conclude that the influence of the three selected variables is similar to the influence on phenolic content; all independent variables positively influence the antioxidant activity of the extracts. Thus, of the selected factors, the temperature is below the limit of statistical significance (microwave power p=0.0040, time p=0.0042). The mixed effect of these variables are presented in figures 8, 9 and 10.

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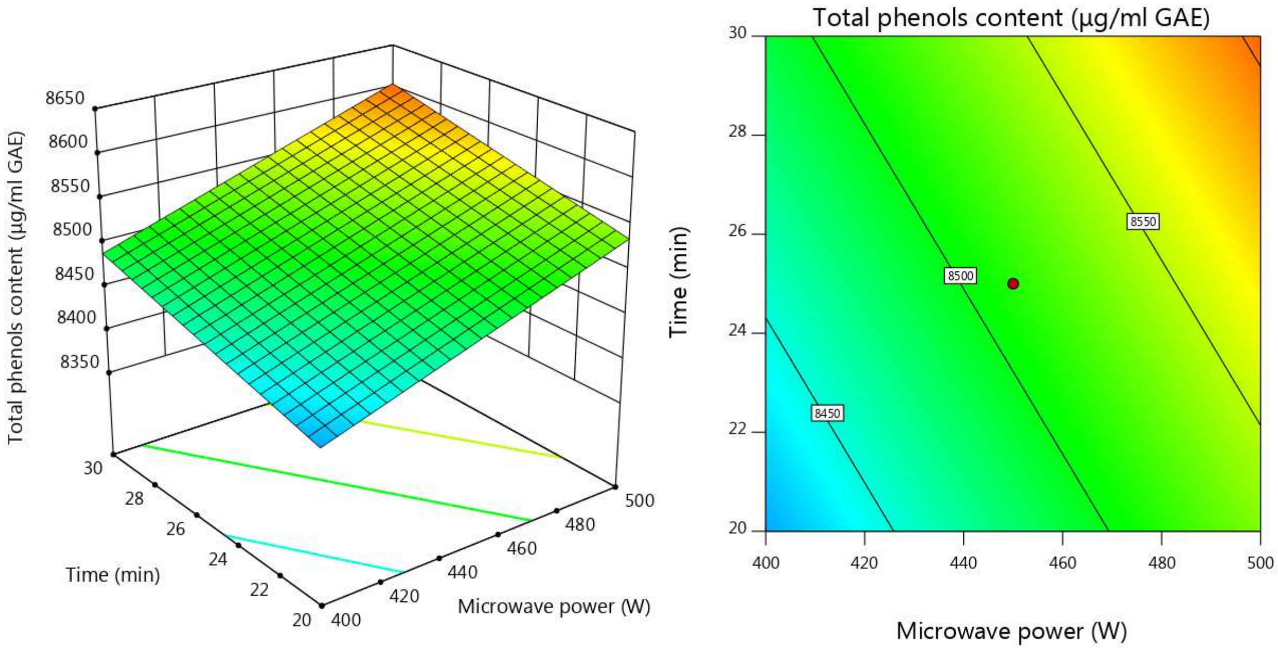

Figure 5. Response surfaces for the total phenols content in solvent-free microwave extraction method (3D - left and 2D - right)

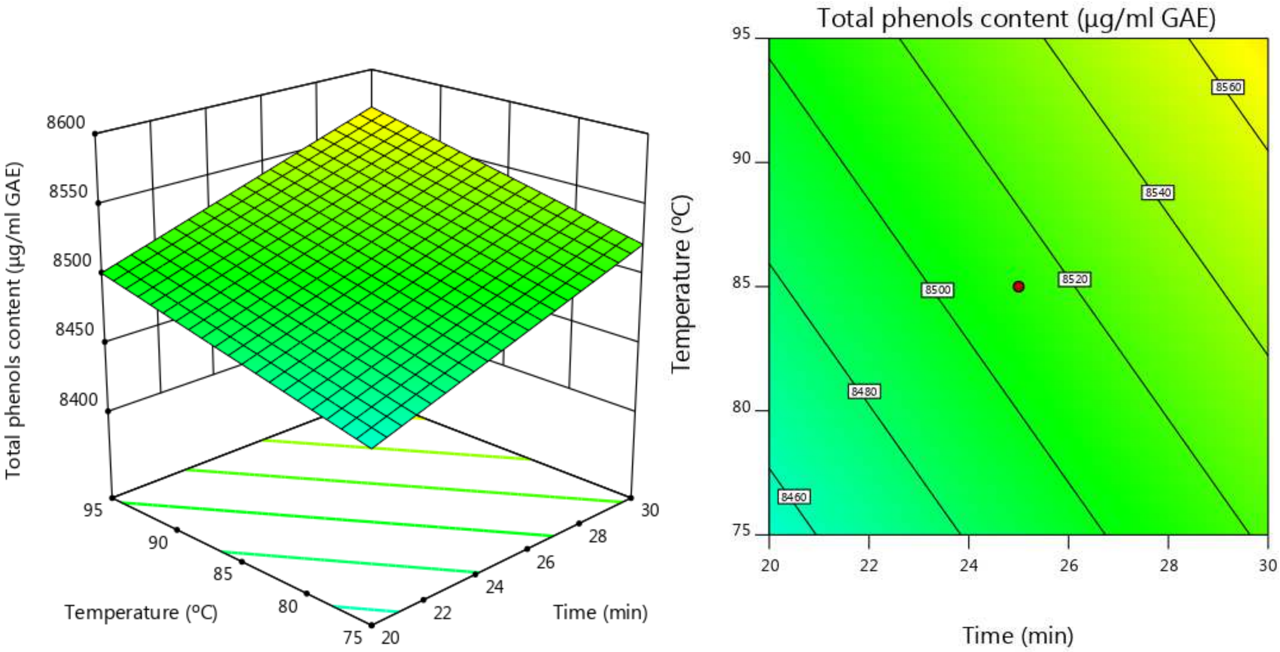

Figure 6. Response surfaces for the total phenols content in solvent-free microwave extraction method (3D - left and 2D - right)

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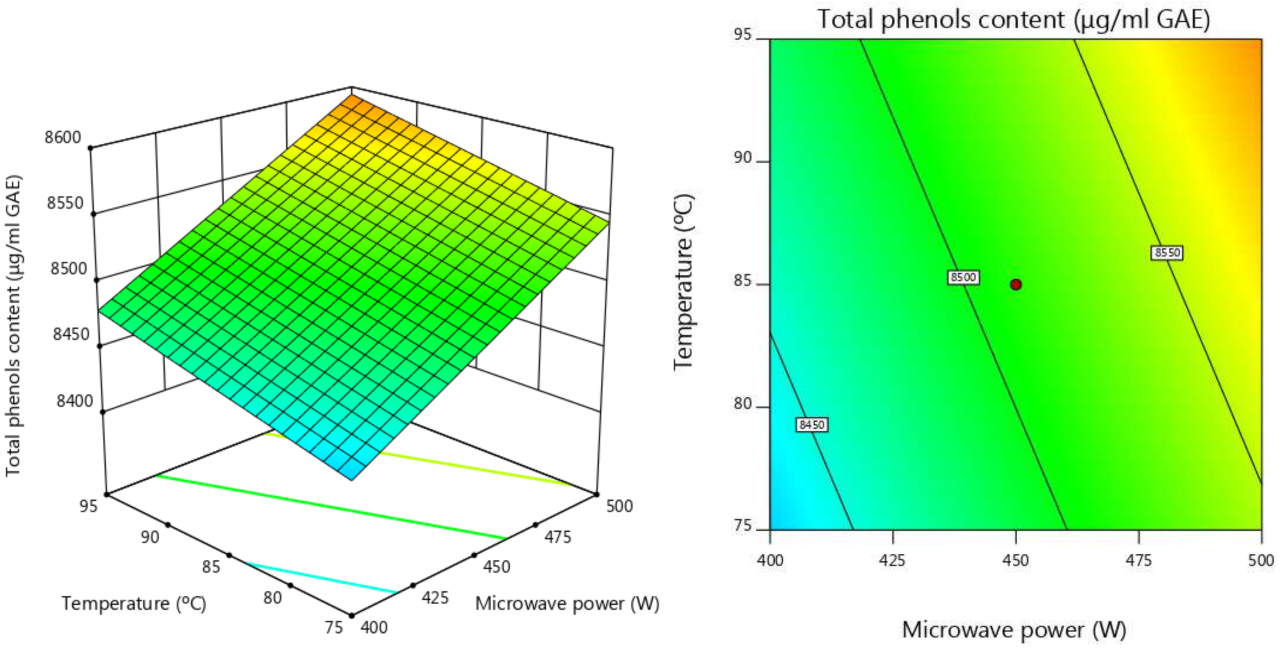

Figure 7. Response surfaces for the total phenols content in solvent-free microwave extraction method (3D - left and 2D - right)

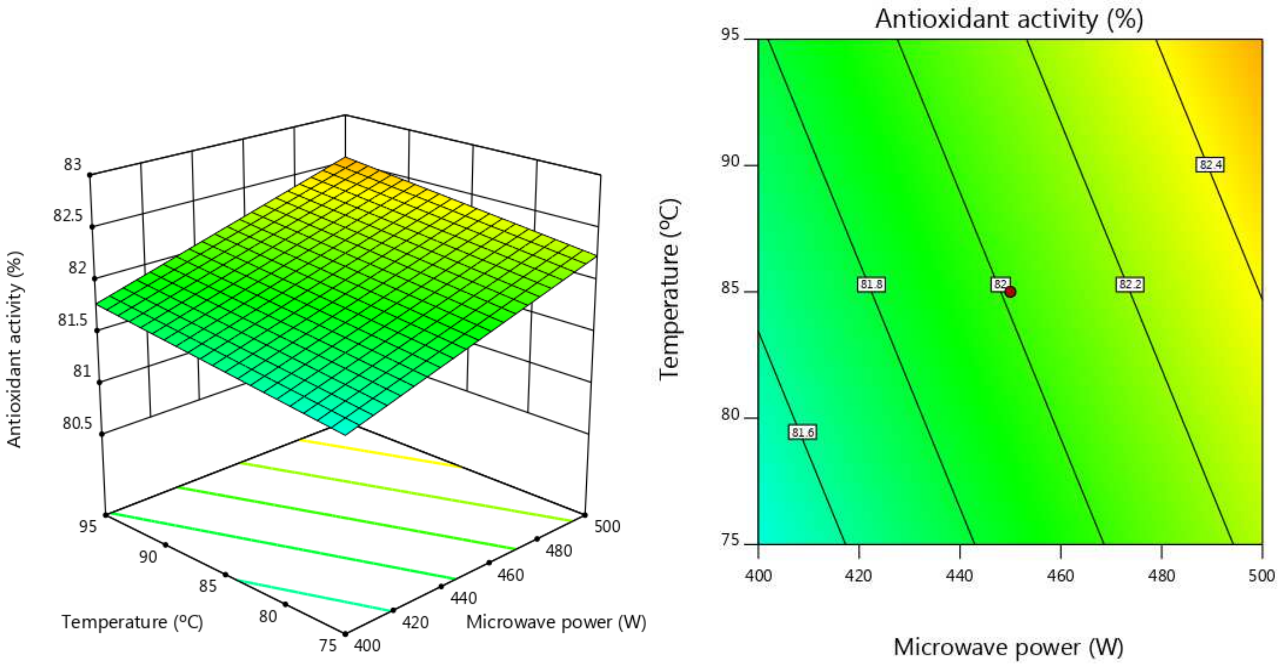

Figure 8. Response surfaces for the antioxidant activity in solvent-free microwave extraction method (3D - left and 2D - right)

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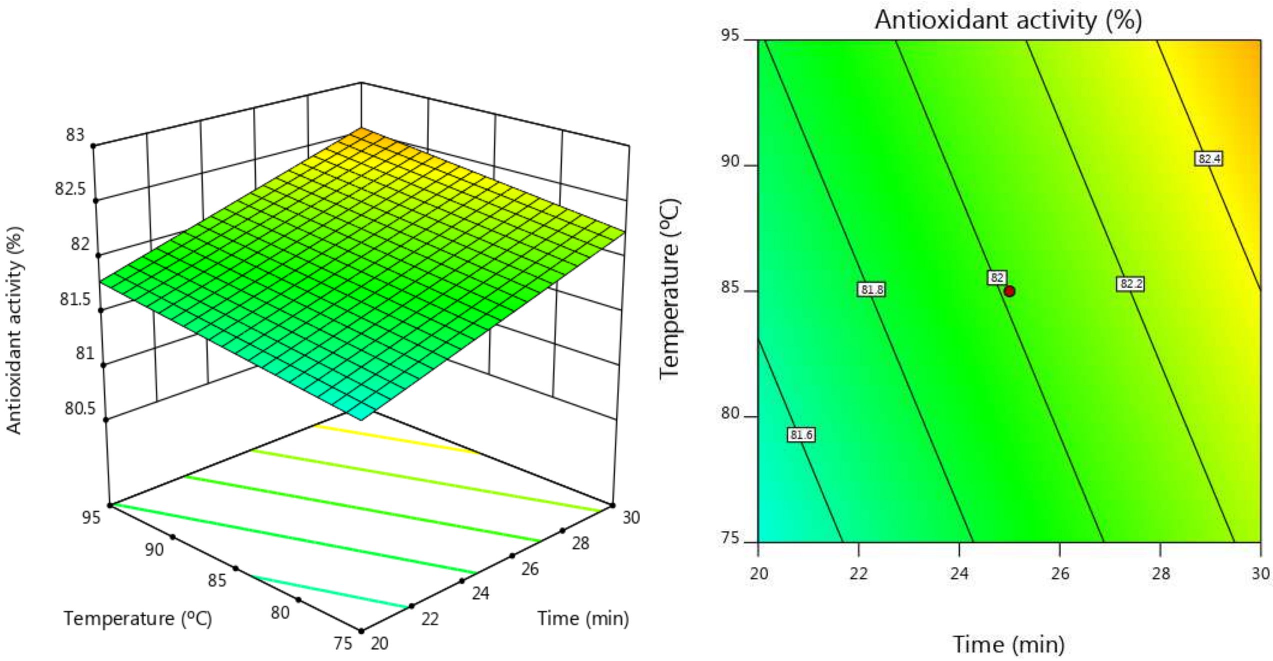

Figure 9. Response surfaces for the antioxidant activity in solvent-free microwave extraction method (3D - left and 2D - right)

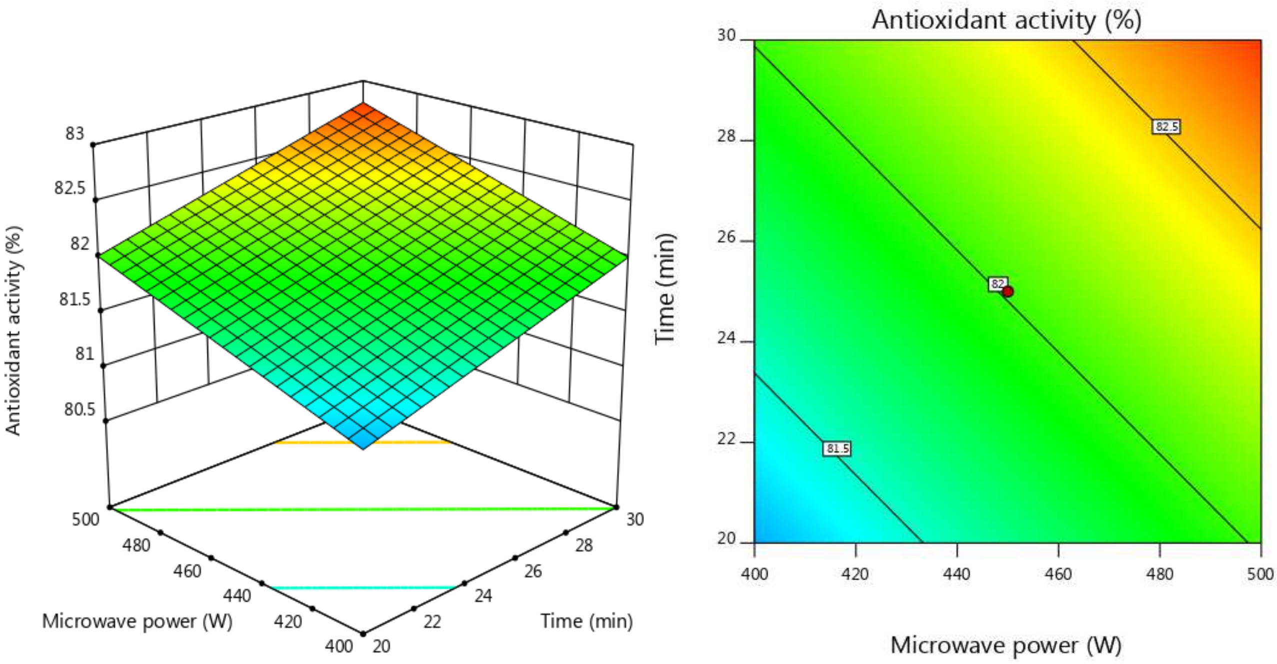

Figure 10. Response surfaces for the antioxidant activity in solvent-free microwave extraction method (3D - left and 2D - right)

To optimize the extraction by this method it was chosen that the total phenol content and antioxidant activity be maximal, and the optimal identified parameters are: microwave power 500W, extraction time 30 minutes and extraction temperature 95^{0}C. These parameters induced 8628.5 µg/ml GAE total phenols content and 82.95% antioxidant activity.

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4. CONCLUSIONS

The optimization methods chosen showed us favorable results adaptable for every extraction method.

Following these analyzes, we can conclude that the antioxidant property of extracts in this plant is best optimized by the microwave assisted by gravity hydrodiffusion method, instead the total phenol concentration is best optimized by solvent-free microwave extraction.

For this medicinal and aromatic plant, the obtained results were optimal.

5. ACKNOWLEDGEMENTS

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