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ORIGINAL ARTICLE

Optimization of the hydroalcoholic extraction process of oregano (*Origanum vulgare* L.)

Optimización del proceso de extracción hidroalcohólica del orégano (*Origanum vulgare* L.)

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Abstract The present study aimed to optimize the hydroalcoholic extraction process of raw material from oregano plants. To achieve this, the plant was dehydrated in an oven at 40 °C for 4 hours, reaching a final moisture content of 12.09%, measured with a thermo-balance. A total of 19 experimental runs were established using the Design Expert 8.0.6 software, varying factors such as extraction time (6, 15, and 24 hours), temperature (30 and 60 °C), and mass/solvent ratio (1:10 and 1:20). Phytochemical analysis revealed that oregano contains flavonoids, triterpenes, quinones, saponins, alkaloids, and phenolic compounds, with the latter being the most abundant. The hydroalcoholic extraction determined the total polyphenol content and antioxidant capacity of each run. The optimized extract showed an antioxidant capacity of 10,491.4 mg/L, higher than the total polyphenol content of 100.814 mg/L. Comparing these results with the numerical optimization, the antioxidant capacity aligned with the model, while the total polyphenol content was lower than the predicted value.

Keywords antioxidant capacity, alkaloids, flavonoids, optimization, polyphenols.

Resumen El presente estudio tuvo como objetivo optimizar el proceso de extracción hidroalcohólica de la droga cruda a partir de la planta de orégano. Para ello, la planta se deshidrató en una estufa a 40 °C durante 4 horas, obteniendo una humedad final del 12,09 %, medida con una termo-balanza. Se establecieron 19 corridas experimentales utilizando el software Design Expert 8.0.6, variando factores como el tiempo de extracción (6, 15 y 24 horas), la temperatura (30 y 60°C), y la relación masa/disolvente (1:10 y 1:20). Según el análisis fitoquímico, el orégano contiene flavonoides, triterpenos, quininas, saponinas, alcaloides y compuestos fenólicos, siendo estos últimos predominantes. Al realizar la extracción hidroalcohólica, se determinó el contenido de polifenoles totales y la capacidad antioxidante de cada corrida. El extracto optimizado presentó una capacidad antioxidante de 10,491.4 mg/L, valor superior al contenido de polifenoles totales de 100.814 mg/L. Al comparar estos resultados con la optimización numérica, se encontró que la capacidad antioxidante se ajustaba al modelo, mientras que el contenido de polifenoles totales fue inferior al valor predicho.

Palabras clave actividad antioxidante, alcaloides, flavonoides, optimización, polifenoles.

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PUBLIS EDITORIAL

Introduction

The food industry faces the challenge of developing high-quality foods that maintain nutritional characteristics and safety over prolonged storage periods. A promising alternative to achieve this is through plant extracts as natural preservatives. However, using these extracts can alter the organoleptic properties of foods, introducing flavors and aromas that are not desirable compared to synthetic preservatives and antioxidants (Nieto, 2020).

The hydroalcoholic extract of oregano (*Origanum vulgare* L.) has gained attention due to its high total polyphenol content and notable antioxidant capacity, making it a candidate for extending the shelf life of foods without compromising their physicochemical and organoleptic quality. Its potential as a natural preservative increases when technologies such as microencapsulation are employed, allowing for preserving its antioxidant and bioactive properties for food applications (Calderón-Oliver & Ponce-Alquicira, 2022).

Antioxidants, both natural and synthetic, play a key role in reducing the adverse effects of reactive oxygen species, which can cause degenerative diseases. In this regard, interest has grown in utilizing natural antioxidants, such as polyphenols, found in various medicinal plants (Ashok et al., 2022). This approach has led to an increase in the demand for food products containing natural additives, offering a healthier alternative to chemical preservatives, whose long-term effects on consumer health are a concern.

Oregano (*O. vulgare* L.) is known for its richness in bioactive substances, including polyphenols with antioxidant properties, making it a valuable resource in the food industry as a natural preservative. These compounds can help replace synthetic additives, improving the safety and quality of food products while minimizing the adverse effects of chemical preservatives. The research aimed to optimize the hydroalcoholic extraction process of oregano based on its total polyphenol content and antioxidant capacity.

Materials and methods

The method for preparing the oregano extract was carried out as follows. First, the oregano stems and leaves were inspected to ensure they were in good condition. Leaves were selected based on homogenous characteristics concerning the vegetative state, size, color, and absence of visible spots, cracks, morphological alterations, or infestations by fungi and parasites. The fresh leaves were dried at 40 °C in an oven without forced air circulation. Once dried, they were ground using a manual grinder and stored in Ziploc double-sealed bags, which were kept in a desiccator until further analysis. The extracts were obtained through maceration with occasional stirring; at the end of each extraction run, the resulting mixture was filtered, and the solid residue was discarded. Finally, the oregano sample underwent various phytochemical analyses to determine its chemical composition.

The determination of total polyphenol content was carried out in several stages. First, the pH was measured following the protocol established by Vázquez-Blanco et al. (2018). Then, the moisture content of the material was evaluated using the method described by Tirado et al. (2015). Finally, the FRAP assay was conducted to determine the total polyphenols, allowing for the quantification of antioxidant capacity in the analyzed extracts.

The experimental design and processing of hydroalcoholic oregano extracts were performed using the Design Expert 8.0.6 program to select the extract with the highest total polyphenol yield and antioxidant capacity. A numerical optimization method was used through a response surface design (IV Optimal), generating a mathematical model describing the variations of variables in each extract. The factors evaluated were ethanol percentage (A), extraction time (B), mass/solvent ratio (C), and temperature (D), while the total polyphenol yield and antioxidant capacity were the response variables. The total number of combinations defined by the software was 19 runs, including 3 replicates.

The experimental conditions used in the study included ethanol concentrations of 60, 75, and 90%, treated for 6, 15, and 24 hours, also expressed in numerical format. The temperature was maintained at nominal levels of 30 and 60 °C. Additionally, a mass/solvent ratio of 1:10 and 1:20 was established. Table 1 shows the experimental runs provided by the program.

Results and discussion

The raw material was subjected to chemical tests to detect bioactive components, including antioxidants, total polyphenols, alkaloids, and phenolic compounds, as detailed in Table 2.

The table shows the presence of phytochemical compounds, highlighting that the ethanolic and aqueous extracts contain phenolic compounds, while the ether extract did not show their presence. Pereira et al. (2009) identified the presence of quinones, benzoquinones, and free amino acids, indicating the presence of fatty compounds in an extract. The detection of these compounds was considered positive if red drops or a colored film appeared in the liquid or on the walls of the test tube. Before conducting the analyses, the image displays the aqueous extracts and the presence of alkaloids and terpenes, as well as unidentified metabolites during the phytochemical screening, which may be due to a possible reduction in their concentration during the drying of the plant. The Dragendorff test for the ether extract revealed

Table 1. Representation of experimental runs

Run	Ethanol (%)	Time (h)	Temperature (ºC)	Drug/solvent ratio
1	60	15	30	1
1	60	15	30	1
1	60	15	30	1
2	75	15	60	2
2	75	15	60	2
2	75	15	60	2
3	60	24	30	2
3	60	24	30	2
3	60	24	30	2
4	90	6	30	1
4	90	6	30	1
4	90	6	30	1
5	60	15	30	1
5	60	15	30	1
5	60	15	30	1
6	60	15	60	2
6	60	15	60	2
6	60	15	60	2
7	75	24	30	1
7	75	24	30	1
7	75	24	30	1
8	75	15	60	2
8	75	15	60	2
8	75	15	60	2
9	75	15	60	1
9	75	15	60	1
9	75	15	60	1
10	90	6	60	2
10	90	6	60	2
10	90	6	60	2
11	90	15	30	2
11	90	15	30	2
11	90	15	30	2
12	60	6	30	2
12	60	6	30	2
12	60	6	30	2
13	60	24	60	1
13	60	24	60	1
13	60	24	60	1
14	90	24	30	1
14	90	24	30	1
14	90	24	30	1
15	75	15	30	2
15	75	15	30	2
15	75	15	30	2
16	90	24	60	2
16	90	24	60	2
16	90	24	60	2
17	90	15	60	1
17	90	15	60	1
17	90	15	60	1
18	75	6	30	2
18	75	6	30	2
18	75	6	30	2
19	75	15	60	1
19	75	15	60	1
19	75	15	60	1

Table 2. Phytochemical profile of oregano

Metabolite	Test	Ether extract	Ethanolic extract	Aqueous extract
Fatty compounds	Sudan	+++		
Alkaloids	Dragendorff	-	++	-
Lactonic grouping	Baljet	-	+++	
Triterpenes/steroids	Lieberman-Burchard	+++	-	
Catechins	Catechins		+++	
Resins	Resins		-	
Reducing sugars	Fehling		+++	+++
Saponins	Foam		-	+-
Phenolic compounds	Ferric chloride (III)		+++	+++
Free amino acids/amines	Ninhydrin		+++	
Quinones/benzoquinones	Bromothymol blue		+++	
Flavonoids	Shinoda		+++	+++
Cardiotonic glycosides	Kedde		-	
Anthocyanins	Anthocyanidins		+-	
Mucilages	Mucilages			+-
Bitter principles	Bitter principles			+++

+: Presence, ±: Regular, -: Absence.

residues of dry material (not precipitated) on the walls of the test tubes. In contrast, the ethanolic extract showed a distinct turbidity.

The test with ferric chloride indicated that the phenolic compounds in the hydroalcoholic extract are derived from pyrocatechol, exhibiting a deep green coloration. Meanwhile, the aqueous extract showed compounds derived from pyrogallol, characterized by an intense dark blue color.

The phytochemical profile of the crude extract revealed that the leaves and stems are a rich source of antioxidants and total phenolics, particularly simple bioactive compounds widely distributed in the plant kingdom. The Baljet test showed that the ethanolic extract contained more triterpenes and steroids, while no significance was observed in the ether and aqueous extracts.

The evaluation of the ether, ethanolic, and aqueous extracts did not show the presence of resins. Similar colorations were observed in the extracts, except in the third test, which exhibited a more intense coloration.

According to the phytochemical assays, when the different extracts were subjected to ferric chloride (III) reagent, a higher presence of phenolic compounds was observed in the ethanolic and aqueous extracts, while no such compounds were detected in the ether extract.

The identification of these secondary metabolites was performed using the Shidona test (Zn/HCl), where the magnesium reaction in an acidic medium reduces flavonoids, generating a color that ranges from reddish-orange to a dark violet hue. In this case, the ethanolic and aqueous extracts showed greater significance, while the ether extract did not present any flavonoids, although a slight precipitation was observed in each sample.

The alcoholic degree has a significant effect on the extraction of compounds present in oregano (*O. vulgare* L.). Subjecting the crude drug to a hydroalcoholic solution allows for determining the presence of these compounds, as well as their antioxidant capacity and total phenolic content in different ethanol concentrations (60, 75, and 90%) for each sample (Alvis, 2012). Considering the presence of compounds in both the aqueous and alcoholic extracts, the effectiveness of the process was evaluated based on total phenolics and antioxidant capacity (Rodríguez et al., 2022).

The analysis of variance performed on the coefficients of response variables related to total phenolic content revealed that the quadratic model was significant at a 95% confidence level. This suggests a statistically significant relationship between mass/solvent and extraction time. Additionally, the coefficient of determination (R^2) indicated that the model explained 96.04% of the variability in total phenolic content (Table 3).

In the analysis of variance for antioxidant capacity, the model was also significant with a 95% confidence level, indicating a statistically significant relationship between the interaction of the factors and the dependent variable of the model. In this case, the R^2 showed a significance of 98.91% of the variability in the antioxidant capacity present in the plant (Table 3).

Table 3. Analysis of variance for the total phenolic content and antioxidant capacity of the hydroalcoholic extract of oregano

Variable	Source	*p*-value
Total phenolic content	Model	0.003
	A	7.84
	B	24.34
	AB	4.7913
	A^2	1.107
	B^2	34.33
	R^2	0.9604
Antioxidant capacity	Model	0.017
	A	893.2638
	B	358.8194
	AB	741.5972
	R^2	0.9891

According to the results, the mass/solvent ratio and extraction time, along with their homologous quadratic terms, showed significant differences in their interactions. Studentized residuals are used as indicators of normality in the distribution of errors in a regression model. They are obtained by dividing the residuals (differences between observed and predicted values) by an estimate of their standard deviation. Upon examining the distribution of the studentized residuals, it was observed that the data fit a normal distribution, indicating that the models for the total polyphenol content and antioxidant capacity were adequate, and the inferences drawn from them are valid. Figure 1 illustrates the relationship between extraction time and ethanol concentration, demonstrating a total polyphenol content higher than the optimized value. The optimal temperature for extraction is 60 °C, with a mass/solvent ratio of 1:10.

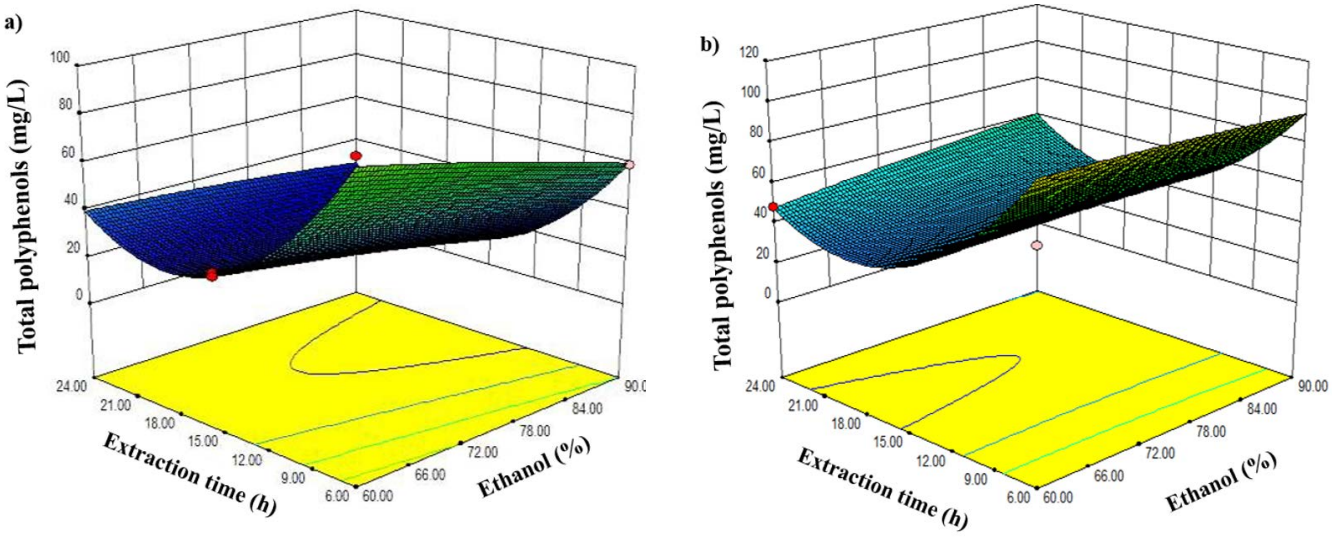

Figure 1. Influence of extraction time and ethanol concentration on total polyphenols: a) 30 °C, b) 60 °C.

Martínez-Flores et al. (2021) noted that the effects of temperature and ethanol concentration were similar. In Figure 2, it can be observed that the optimal points for the extraction of total polyphenols varied, allowing for the determination of the optimal polyphenol value based on the mass/solvent ratio with the percentage of ethanol diluted in 1 gram of crude oregano.

Figure 2 shows that the antioxidant capacity reached its highest level of 6325.46 mg/L with an ethanol concentration of 75% and an extraction time of 15 hours. This value was obtained at a temperature of 30 °C and a crude drug/solvent ratio of 1:10. At 60 °C, an antioxidant capacity value of 6325.46 mg/L was obtained, which is higher than predicted.

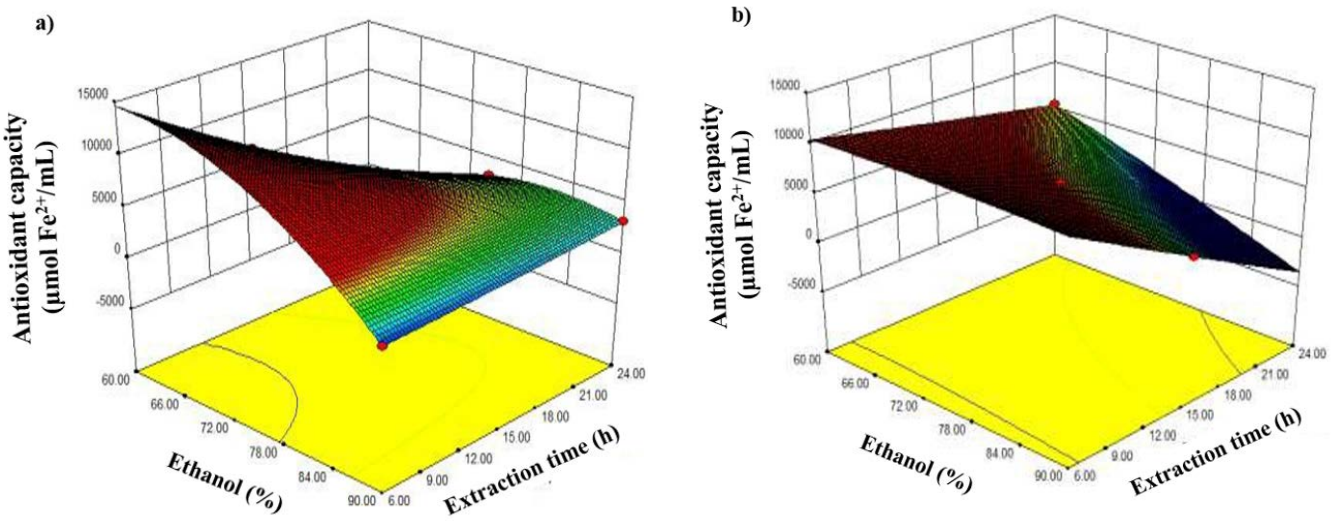

Figure 2. Influence of extraction time and ethanol concentration on antioxidant capacity: a) 30 °C, b) 60 °C.

For the numerical optimization of the extraction process, previously evaluated intervals of the mass/solvent ratio and extraction time were used. This allowed for higher final results regarding total polyphenols and the antioxidant capacity present in the crude oregano.

The numerical optimization of the extraction process was carried out according to pre-established parameters. An interval for the mass/solvent ratio was defined between 1.0 and 2.0, and the extraction time was set in the range of 6 to 24 hours. To maximize total polyphenols, limits between 25.7625 and 124.88333 mg/L were considered. Additionally, the goal was to maximize antioxidant capacity within a range of 2099.4444 to 6325.4629 µmol Fe^{2+}/mL. Together, these parameters provide a framework for optimizing the process, ensuring that they remain within the established limits and achieving optimal results in the total polyphenols extraction and antioxidant capacity.

The results indicated the optimal parameters for the extraction of bioactive compounds. The mass/solvent ratio was established at 1:10, suggesting an appropriate proportion to maximize the extraction of the desired components. The extraction time was fixed at 6 hours, a period considered efficient for achieving a significant recovery of the metabolites. The results showed a total polyphenol content of 100.814 mg/L, reflecting a considerable amount of these antioxidant compounds in the extracted sample.

The antioxidant capacity reached a value of 10,491.4, indicating the strong antioxidant capacity of the extract. Finally, the statistical convenience (0.87015782) suggested that the model used to optimize these parameters is adequate and that the results obtained are reliable. These data provide a solid foundation for future research on the antioxidant potential of the analyzed crude drug.

To obtain the optimized extract, the mass/solvent ratio was established using 1 g of crude drug with 10 ml of 60% ethanol diluted in distilled water, for 6 hours at 60 °C. The optimized extract presented a content of 0.111 mg/L of total polyphenols, a value lower than theoretical. This is attributed to the different dilutions performed on the extract, which modified the concentration of polyphenols in the hydroalcoholic extract of the crude oregano (de Torre et al., 2020).

The Design Expert 8.0 program estimated an antioxidant capacity of 10,491.4 mg/L. When comparing this result using spectrophotometry, a value of 10,340.55 mg/L was obtained, demonstrating that the difference between the theoretical and practical value is minimal, aligning with the mathematical model.

Conclusions

The drying process of oregano leaves and stems was carried out for 48 hours at a temperature of 40 °C, resulting in leaves with a final moisture content of 12%, which matched the desired moisture indicator. During the extraction of the hydroalcoholic extract from the crude drug, several key compounds were identified, such as total polyphenols and antioxidant capacity, in addition to detecting the presence of quinones, alkaloids, triterpenes, saponins, phenolic compounds, and flavonoids through phytochemical analysis. The optimized hydroalcoholic extract showed a concentration of total polyphenols of approximately 100 mg/L, a value obtained through numerical optimization, while the antioxidant capacity was higher than expected, exceeding 10,000 mg/L in the laboratory. Finally, the phytochemical analysis using various assays such as Shidona, foam, and ferric chloride (III) allowed for determining the presence of metabolites in the three extracts analyzed: aqueous, ethereal, and ethanolic.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Sixto A. Gavilanez and Jaime O. Rojas: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, drafting the original manuscript and writing, review, and editing.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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