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Original Article

Kinetic evaluation of ultrasound and microwave pretreatments for flavonoids (pinocembrin and galangin) extraction from the residual bagasse of Mexican oregano (*Lippia graveolens Kunth*)

Evaluación cinética del pretratamiento con ultrasonido y microondas para la extracción de flavonoides (pinocembrina y galangina) a partir del bagazo residual del orégano mexicano (*Lippia graveolens Kunth*).

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ABSTRACT

The essential oil profile of Mexican oregano (*Lippia graveolens* Kunth) makes it a spice with significant economic potential. However, its high concentration of water-soluble phenolic compounds (pinocembrin and galangin), flavonoids with important therapeutic properties, is little used. The content of these flavonoids remains in significant quantities in the residual bagasse obtained after the essential oil extraction process. This bagasse, being rarely used and due to its inadequate disposal, represents a source of contamination. This research focused on taking advantage of the residual bagasse, extracting the flavonoid compounds (pinocembrin and galangin) and analyzing by HPLC-DAD. As extraction pretreatments, the use of ultrasound and microwave at different powers was evaluated, followed by extraction with agitated maceration. The extraction kinetics could be adjusted to a second-order model, revealing a two-stage extraction process: diffusion and washing. The maximum extraction capacity (Cs) and recovery of pinocembrin and galangin were obtained using ultrasound at 325 W for 5 min, reducing the total extraction time by 59 %. The total phenolic content (Folin-Ciocalteu) and antioxidant capacity (ORAC, DPPH, FRAP) of the extracts obtained were evaluated. This approach represents an alternative use of residual bagasse and a significant improvement in the efficiency of the conventional extractive process.

Keywords: Valorization; extraction kinetic model; pinocembrin, galangin.

RESUMEN

El perfil del aceite esencial del orégano mexicano (*Lippia graveolens Kunth*) lo convierte en una especia con importante potencial económico. Sin embargo, es poco aprovechada su alta concentración en compuestos fenólicos hidrosolubles (pinocembrina, galangina), flavonoides con importantes propiedades terapéuticas. El contenido de estos flavonoides permanece en cantidades significativas en el bagazo residual obtenido tras extraer el aceite esencial. Este bagazo es poco utilizado y por su inadecuada eliminación representa una fuente de contaminación. Esta investigación se enfocó en aprovechar el bagazo residual, extrayendo los compuestos flavonoides (pinocembrina y galangina), analizados mediante HPLC-DAD. Se evaluaron dos pretratamientos de extracción: ultrasonido y microondas, empleando diferentes potencias. Seguido de la extracción mediante maceración agitada. Las cinéticas de extracción ajustadas a un modelo de segundo orden revelaron un proceso de extracción de dos etapas: difusión y lavado. La máxima capacidad de extracción (Cs) y recuperación de pinocembrina y galangina se obtuvieron empleando ultrasonido a una potencia de 325 W durante 5 min, reduciendo el tiempo de extracción en un 59 %. Se evaluó el contenido de fenoles totales (Folin-Ciocalteau) y capacidad antioxidante (ORAC, DPPH, FRAP) en los extractos obtenidos. Este enfoque representa un uso alternativo del bagazo residual y una mejora significativa en la eficiencia del proceso extractivo convencional.

Palabras clave: Valorización; modelo cinético de extracción; pinocembrina; galangina.

INTRODUCTION

Mexico is the second country with the highest production of oregano worldwide, producing 6 500 tons in 2022, acording to the National Institute of Forestry, Agriculture and Livestock Research (INIFAP, 2024). Oregano is widely distributed and marketed worldwide, since it represents a plant of high commercial value in the international market due to the quality of the components present in the essential oil extracted from the leaves (Mastelić *et al*., 2008). However, during the extraction process of oregano essential oil, a large amount of residual matter also known as "bagasse" is generated, which represents up to 95 % of the initial weight (Martínez-Nataren *et al*., 2012). It has been reported that this residue is rich in pinocembrin ((2 S)-2,3-Dihydro-5,7-dihydroxy-2-phenyl-4-one) and galangin (3,5,7-trihydroxy-2-phenylchromen-4-one) (Cuevas-González *et al*., 2021; Arias *et al*., 2020), flavanones with documented bioactive properties, including anticancer, antimicrobial, anti-inflammatory, antioxidant and protective

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activities against cerebral ischemia (Chung *et al*., 2011; Tao *et al*., 2018; Guan *et al*., 2021). Regarding pinocembrin, it is included in patent applications as a medication for the management of pulmonary fibrosis and acute intracerebral hemorrhage (Du *et al*., 2018; Cheng *et al*., 2019).

However, studies for pinocembrin and galangin recovery from the residual bagasse of Mexican oregano are scarce and very limited, reporting a lengthy extraction process, the requirement for expensive solvents in large amounts, the poor extraction selectivity, and the potential decomposition or thermolabile chemicals, are the key obstacles. Some authors (Oreopoulou *et al*., 2020; Cid *et al*., 2021; Arias *et al*., 2020) have already studied the residual bagasse of some types of oregano. Arias *et al*. (2020) evaluated supercritical conditions of *L. origanoides* (Verbenaceae) harvested in Santander, Colombia, where supercritical CO_2 allowed the extraction of pinocembrin and galangin. On the other hand, Cid *et al*. (2021) investigated the *Poliomintha longiflora* variety from Chihuahua, Mexico, focusing on the quantification of antioxidant capacity and total phenolics. The aforementioned authors conclude that the residual bagasse obtained after the oregano essential oil extraction process, can become an important source of bioactive molecules. In addition, the use of this waste could also significantly reduce the environmental impact it generates. Therefore, the need to maximise the use of this residual bagasse and the bioactive compounds it presents becomes evident, by optimizing and implementing an extraccion pretreatment that contributes to greater selectivity of compounds and reduction of time, energy and solvent.

Conventional extraction methods, such as distillation and maceration, in big scale and laboratory level, can be characterized by their slowness and low productivity, which can lead to the loss of bioactivity due to the chemical degradation reactions that occur (Bajkacz *et al*., 2018; Ali *et al*., 2019). Innovative extraction methods mostly center on the use of ultrasound (UAE) and microwaves (MAE), which can significantly improve extraction efficiency, reduce processing times (Chemat *et al*., 2017; Liao *et al*., 2021; Zhi-Ting *et al*., 2023), and, above all, show certain extraction specificity as shown in the research conducted by Michalaki *et al.* (2023), who optimized the ultrasound extraction of phenolic compounds from oregano (*Origanum vulgare* ssp. hirtum). However, in this study is of interest to apply these technologies as a pretreatment in the maceration process to impprove the extraction process.

Therefore, the objective of this study was to valorize the residual bagasse through the efficient extraction of pinocembrin and galangin, optimizing a pretreatment that would allow to improve its recovery. The kinetic mechanisms and pretreatment parameters (UAE and MAE) were evaluated in order to model the extraction behavior, minimizing the time required and favoring the selectivity of the compounds during the process. With this approach, we seek to establish effective processes for the valorization of flavonoids present in Mexican oregano residues, which can serve as a basis for future research and open new opportunities in the pharmaceutical and food industries, taking advantage of their bioactive properties.

MATERIAL AND METHODS

Vegetal material

Plant material was obtained from the municipality of Villa Guerrero, Northern region of the state of Jalisco, México (coordinates 103º22'30" at 103°50'00" west longitude and 21°54'00" at 22º10'00" north latitude; at 1,767 meters above sea level). The species of *Lippia graveolens* Kunth was identified with specimens from the CREG Herbarium of the Technological Institute of Tlajomulco, Jalisco. The proportion of leaves, fruit and stems was determined. Oregano plant was subjected to steam distillation for 3 hours to separate the essential oil (Soto-Armenta *et al*., 2017). The residual oregano bagasse was dried (humidity 8-10 % w/w) in a tray dryer (GL-70 A) at 35 °C for 24 hours.

Ultrasound assisted extraction (UAE)

An ultrasound system (20-25 KHZ, 650 W, SCIENTZ-JY88-II (N), S/N 01C1005) with a 10 mm diameter probe was used. The probe was immersed 20 mm into the sample, which contained ground residual bagasse (0.4 mm) and a hydroalcoholic solution (58 % ethanol v/v), with a bagasse-solvent ratio of 1:20 (w/v), conditions previously optimized by Flores-Martínez *et al.* (2016) for the extraction of phenolic compounds present in Mexican oregano (*Lippia graveolens* Kunth). Kinetics were carried out at ultrasonic powers of 30, 50 and 70 % corresponding to 195, 325 and 455 W respectively. These values were selected according to preliminary tests. Samples were taken every minute for a total time of 5 m for each of the powers, filtered and kept until analysis at 4 °C in amber glass flasks.

Microwave assisted extraction (MAE)

It was carried out in a microwave oven (NN-CF778BPQ, Panasonic). The ground residual bagasse (0.4 mm) was placed in a beaker with a hydroalcoholic solution (ethanol 58 % v/v), ratio 1:20 (w/v). Kinetics were carried out at different powers 10, 30 and 70 %, corresponding to 70, 120 and 350 W respectively. These values were selected according to preliminary tests. Samples were taken every minute for a total irradiation time of 5 m. Samples were filtered and kept until analysis at 4 °C in amber glass flasks.

Hydroethanolic extraction by maceration

The extraction of flavonoids was carried out using the maceration method according to the optimal conditions described by Flores-Martínez *et al*. (2016). The operating system has reflux to avoid losses due to evaporation. The solid waste (Mexican oregano without essential oil) was subjected to grinding and sieving, with the purpose of obtaining the desired granulometry (0.4 mm) and thus facilitating the extraction process. The extraction was carried out in a 500

mL balloon flask, with a solute-solvent ratio of 1:20, using 58 % ethanol as solvent. The extraction time was 1 h with a temperature of 75 °C and controlled stirring of 300 rpm on a stir plate. At the end of the extraction, the extract was filtered and the volume measured. The kinetics carried out in this extraction process were carried out in a total time of 60 m, where samples were taken at 10 m intervals.

Optimal pretreatment determination

The results obtained in the extraction kinetics were adequately adjusted to a second-order model (Equation 1). The kinetic constants (k_2), extraction capacity (Cs) and correlation coefficient (R^2) were determined experimentally by applying the second-order extraction model described by Ho *et al*. (2005) and Qiu *et al*. (2009), where Cs and Ct were the concentrations of pinocembrin (mg/mL Ext) and galangin from the residual oregano bagasse at equilibrium conditions and at any time "t", respectively. The second-order kinetic equation is described by Equation (1):

$$\frac{dCt}{t} = (k_2)\ (Cs - Ct)^2 \quad (1)$$

where k_2 (1/mgPin*min) is the second-order extraction rate constant. The extraction speed (Ct/t) can be obtained by the inverse of the previous equation and the initial extraction rate h (equivalent to Ct/t when t approaches 0) can be defined as $h = k_2 Cs$, in such a way that the previous equation can be expressed according to Equation (2). The equation is presented in linear form, where *t/Ct* is the dependent variable, 1/Cs is the ordinate at the origin and 1/h represents the slope coefficient.

$$\frac{t}{Ct} = \frac{1}{h} + \frac{t}{Cs} \quad (2)$$

In this way, the initial extraction rate h, the extraction capacity Cs and the second-order extraction rate constant can be determined experimentally from the slope and the intercept at the origin by plotting t/Ct against t.

Total Phenols determination

The quantification of total phenolic compounds was carried out using the Folin-Ciocalteu method (Cortés-Chitala *et al*., 2021). For this, the extracts were diluted at a 10 % proportion in 60 % ethanol solution.

Fifty µL of solution were taken and mixed with 0.5 mL of 0.67 N Folin reagent and 0.5 mL of a Na_2O_3 solution at a concentration of 1.9 M. The reaction was carried out at room temperature in the dark for 1 h. Subsequently, the samples were measured spectrophotometrically at a wavelength of 760 nm. Gallic acid was used as a standard reference. The results are expressed as mg equivalent of gallic acid by mL (mg GAE /mL).

Antioxidant Capacity by DPPH

The antioxidant capacity of the extracts was evaluated using visible spectrophotometry in the presence of the radical 1,1-diphenyl-2-picrylhydrazine (DPPH) (Cortés-Chitala *et al*., 2021). Two mL of 80 % methanol (blank) and 2 mL of the different extracts diluted to 0.1 % were taken, respectively. Then, to each sample, including the blank, 2 mL of a freshly prepared DPPH solution (2.5 mM) were added. Readings of the blank (Abs blank) and samples (Abs sample) were taken after a period of 30 m at 518 nm. The percentage of inhibition was calculated using the following equation:

$$\%\ Inhibition = \frac{Abs\ Blank - Abs\ Sample}{Abs\ Blank} \quad (3)$$

Results were expressed in Trolox equivalents (TxEq) based on a calibration curve previously prepared using known Trolox concentrations (0, 20, 40, 60, 80, 100 mg TxEq/mL).

Antioxidant Capacity by FRAP

The analysis of antioxidant capacity was complemented with the FRAP (Ferric Reducing Antioxidant Power) technique, according to the FRAP kit protocol purchased from Sigma-Aldrich that uses the reagent 2,4,6-tripyridyl-s-triazine (TPTZ). The reduction reaction was measured at a wavelength of 593 nm. A standard curve was prepared using different dilutions of the Fe^{2+} standard (2 mM). The results obtained are expressed in mM Fe^{2+}/mL.

Antioxidant Capacity by ORAC

For the antioxidant capacity determined by the ORAC method (Cortés-Chitala *et al*., 2021), Trolox (antioxidant agent) was used as a reference standard and gallic acid as a positive control. Reactions were performed by mixing 100 µL of 120 nM fluorescein with 20 µL of PBS (blank), Trolox and extracts, respectively. The microplate, along with freshly prepared 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) free radical, was heated at 37 °C for 15 m. Subsequently, 80 µL of AAPH were added to the mixture, and the reaction was measured every 4 m until the fluorescence decreased to less than 10 % of the initial value (approximately 2 h). Three replicates were analyzed for each sample and at each curve level.

Fluorescence values were normalized according to the blank curve (without antioxidant). The area under the fluorescence decay curve (AUC) was calculated from the normalized curves as:

$$AUC = \left(0.5 + {}^{f1}/_{f0} + {}^{f2}/_{f0} + {}^{f3}/_{f0} \ldots {}^{fn}/_{f0}\right) * 4 \quad (4)$$

The AUC_{net}, corresponding to each concentration of Trolox and each sample, was calculated by subtracting the respective AUC minus that corresponding to the blank. The regression equation was calculated based on the AUC_{net} and the corresponding Trolox concentration. ORAC values were expressed as Trolox equivalents/mL using the standard calibration curve regression equation.

Selectivity

Selectivity was determined according to the method described by Psarrou *et al*. (2020). The solids total content in the

extract samples was determined by placing 5 mL of this in previously weighed aluminum trays, then taken to a drying oven at 100 °C. The procedure was performed in triplicate and the selectivity of the extraction was calculated based on the concentration (mg/mL) of flavonoids (pinocembrin and galangin) relative to the total solids content (mg/mL) in the extract. The selectivity is expressed as percentage (%) of selectivity.

Quantitative analysis by HPLC-DAD

The quantification of pinocembrin and galangin present in the extracts was carried out with the high-performance liquid chromatography method coupled to a diode array detector (HPLC-DAD) proposed by Arias *et al*. (2020). Chromatographic separation was performed in a chromatograph (AT, Palo Alto, CA, U.S.A.) coupled to a diode array detector and an autosampler, using a GEMINI C18 column (250 × 4.6 mm inner diameter, 5 µm particle size; Phenomenex, Torrance, CA, USA). Two mobile phases were used: an aqueous solution of 0.5 % v/v formic acid (A) and HPLC grade acetonitrile (B) at a constant flow of 1 mL/min, with the following gradient: 2 – 12 % (B) 0 – 15 min, 12 % (B) 15 – 23 min, 12 – 40 % (B) 23 – 46 min, 40 – 90 % (B) 46 – 71 min, 90 % (B) 71 – 75 min, 90–2 % (B) 75 – 80 min, 2% (B) 80 – 85 min.

The compounds were detected at 245 nm, 270 nm, 290 nm, and 515 nm wavelengths. The extract was diluted in a 1:100 proportion using a solution of 50 % v/v mixture of methanol and water with 0.5 % v/v formic acid. Comparing each reference compounds retention time (tR) and wavelengths of maximum absorbance with those obtained from the chromatograms, the compounds detected were identified.

Statistical analysis

All experiments were performed in triplicate, and the results presented as the mean ± standard deviation. Statistical analysis was performed using ANOVA, and a p-value of 0.05 used to determine significant differences between the means with Tukey's test (Minitab, Inc. State College, Pennsylvania, PA USA).

RESULTS AND DISCUSSION

Physical analysis

A detailed analysis of various characteristics of the oregano sample was conducted, highlighting its origin in Villa Guerrero (OVG), the taxonomic identification as *Lippia graveolens* Kunth, and humidity of 6.94 %. The aerial part was constituted of 66.64 % leaves, 30.80 % fruits and 2.56 % of stem. The essential oil content was recorded at 2.40 %, within the average range characteristic of this plant species, as previously reported (Soto-Armenta *et al*., 2017). Morais *et al*. (2016) reported that the composition and quantity of secondary metabolites found in several plants may be caused by both genetic and edaphoclimatic factors (temperature, light intensity, seasonal effect, soil characteristics, etc.). It should be noted that the majority of aromatic species that have medicinal applications are found in the wild, which allows them to interact strongly with the environment (Santos *et al*., 2016), so it was important to consider the wild state of the plant used in the present work. The proportion of residual bagasse accounted for 76.39 % of the total sample, a part utilized in the present investigation.

Total phenols, flavonoids and antioxidant capacity

The evaluation of total phenols (Table 1) showed a variation between the Mexican oregano extract and its residual bagasse, with values of 5.507 ± 0.107 and 3.697 ± 0.010 mg GAE/mL respectively (equivalent to 110.14 mg/g DW and 73.94 mg/g DW). The oregano total phenols content obtained was higher than those reported from other regions and/or varieties. For example, in the case of Mexican oregano from Huejuquilla, Jalisco, Cortés-Chitala *et al.* (2021) reported a total phenolic content of 4.41 mg GAE/mL, while Oreopoulou *et al.* (2021) reported a content of 49.9 mg GAE/g DW in extracts of *Origanum vulgare ssp. Hirtum*. On the other hand, Cid *et al.* (2019) who have already studied extracts of solid residues of oregano (*Poliomintha longiflora)* presented a value of 1.32 mg EAG/mL. This analysis includes the measurement of flavonoids, tannins and quinones, where variations detected can be attributed to the varying ratios and quantities of these compounds among themselves, since in addition the residual bagasse corresponds to a by-product from which part of said compounds have already been extracted.

The evaluation of the antioxidant capacity through DPPH, FRAP and ORAC assays generally reveals notably higher values in the untreated oregano sample, compared to those obtained for the residual bagasse (Table 1). These results highlight the differences in the antioxidant capacity between the two samples, indicating that these variations

Tabla 1. Análisis de fenoles totales, capacidad antioxidante (DPPH, FRAP y ORAC) y flavonoides (pinocembrina y galangina) en los extractos hidroetanólicos de orégano mexicano y bagazo residual.
Table 1. Analysis of total phenols, antioxidant capacity (DPPH, FRAP and ORAC) and flavonoids (pinocembrin and galangin) in the hydroethanolics extracts of Mexican oregano and residual bagasse.

	Hydroethanolic extracts	
	Mexican oregano	Oregano residual bagasse
FT (mg GAE/mL)	5.507 ± 0.107 [a]	3.697 ± 0.010 [b]
DPPH (mg EqTx/mL)	8.564 ± 0.033 [a]	5.127 ± 0.048 [b]
FRAP (mM Fe2+ Eq)	104.467 ± 0.082 [a]	69.981 ± 0.043 [b]
ORAC (mg EqTx/mL)	0.750 ± 0.065 [a]	0.735 ± 0.087 [b]
Pin (mg/mL)	3.915 ± 0.019 [a]	2.240 ± 0.036 [b]
Gal (mg/mL)	1.848 ± 0.012 [a]	1.032 ± 0.009 [b]

FT: Total phenols, GAE: Gallic acid equivalent, EqTx: Trolox equivalent. Pin: Pinocembrin, Gal: Galangin. The different superscript letters indicated significant differences between samples according to the Tukey test ($p < 0.05$).

could be explained due to the different chemical composition of each material, since it is well known that the essential oil contains compounds with antioxidant capacity, which contributes to the observed differences in the tests. The application of the different techniques for measuring antioxidant activity helps us to realize the different mechanisms that oregano and its residual bagasse can have as an antioxidant agent. The ORAC reaction is based on the transfer of protons, whereas the DPPH method relies on the transfer of electrons. Similar to this, the ORAC test calculates the area under the curve (AUC), which includes the degree and duration of free radical inhibition by an antioxidant or extract at a certain dose. The DPPH test assesses the antioxidant capacity assessed at a specific period. The FRAP technique is based on the reduction of the ferric ion to the ferrous state, where it combines with the chemical 2,4,6-Tripyridyl-s-Triazine (TPTZ) to generate a colorful complex.

The results of the pinocembrin and galangin quantification in the hydroethanolic extracts of Mexican oregano and its residual bagasse, are presented in Table 1. These were determined by identifying the retention times of standard samples. The retention times for pinocembrin and galangin were set at 50.0 and 50.7 m, respectively. The Mexican oregano extract without treatment exhibited a content of 3.915 ± 0.019 mg/mL of pinocembrin and 1.848 ± 0.012 mg/mL of galangin. On the other hand, the residual bagasse revealed a significant disparity in the content of these flavonoids, recording concentrations of 2.240 ± 0.036 mg/mL of pinocembrin, and 1.032 ± 0.009 mg/mL of galangin.

These compounds have already been identified and quantified in oregano and its residual bagasse (Oreopoulou *et al*., 2021; Cortés-Chitala *et al*., 2021; Cid-Pérez *et al*., 2019; Arias *et al*. 2020). For untreated oregano, Cortés-Chitala *et al*. (2021) in hydroethanolic extracts of *L. graveolens*, presented values of 0.020 to 3.321 mg/mL for pinocembrin, and 0.003 to 0.436 mg/mL for galangin. For this same variety, Flores *et al*. (2016) reported a value of 0.574 mg/mL of pinocembrin. While quantification studies of these flavonoids in residual bagasse are scarce, Arias *et al*. (2020) have reported results on the quantification of pinocembrin and galangin in the residual bagasse of *L. origanoides*, and show lower concentrations compared to those obtained in the present investigation. They used Soxhelt extraction and supercritical fluids modified with ethanol, achieving concentrations of 26 mg/g of pinocembrin and 3.9 mg/g of galangin, while in the present investigation concentrations of 78.3 to 44.8 mg Pin/g and 36.96 to 20.64 mg Gal/g. This quantitative analysis allowed us to compare and confirm the potential that residual bagasse presents as a raw material rich in flavonoids, since the essential oil extraction process focuses on the oily part and fat-soluble compounds, leaving the residual bagasse as a rich source of molecules of biological interest, such as polyphenols that are practically insoluble in the oil extraction medium. These flavonoids maintain their stability in this extractive process, since they do not experience significant concentration variations in the untreated oregano and in the residual bagasse. In this sense, since significant quantities of pinocembrin and galangin remain in the distillation, bagasse receives special attention, these flavonoids representing valuable components with potential therapeutic applications.

Effect of extraction pretreatments

Extraction kinetics

The kinetic representation of the pretreatments (ultrasound and microwave) at different powers for the extraction of pinocembrin (Pin) and galangin (Gal) from the residue of Mexican oregano bagasse, is shown in Figure 1 (A and C). In both cases, the initial concentration of pinocembrin experienced a rapid increase until the second minute, followed by a significant decrease in the microwave treatments at 210 and 350 W. The ultrasound treatments at the different powers and microwaves at 70 W showed an increase in accumulation, tending towards an equilibrium with respect to time.

The pinocembrin extraction data obtained through the various pretreatments were fitted to a second-order kinetic model (Table 2). Figure 1B displays the results of t/Ct versus time in a linear form (Eq. 2), where Ct represents the concentration of pinocembrin in relation to extraction time. The second-order models for each pretreatment were adequately adjusted to the experimental extraction process, showing linearity until minute 4, with R^2 values that ranged between 0.995 and 0.972.

For galangin, the pretreatment kinetics showed a rapid increase up to minute one, until an equilibrium with respect to time is reached, with the exception of microwave treatments at powers of 210 and 350 W, since these showed a decrease, exhibiting a degradation of galangin as time passed.

By graphing t/Ct versus the extraction time of each of the pretreatments, Figure 1D was obtained, data that was adjusted to a second-order kinetic model (Table 2), with R^2 values that ranged between 0.914 and 0.999.

It is evident that the experimental results of pinocembrin and galangin extraction generally fit well with a second-order kinetic model. Two extraction stages are observed, an initial rapid stage extending up to minute two, then a stage that is slower leading to a state of concentration equilibrium and, in some cases, compound degradation. Research on the extraction of components from plants has already reported the kinetic extraction of some compounds such as total phenols, alkaloids, among others, showing the same extraction phenomenon characterized by a rapid extraction stage (washing), followed by a slower stage (diffusion) (Chung-Hung *et al*., 2014; Psarrou *et al*., 2020). When a second-order model represents an extraction process, it is characterized by the rapid removal of more soluble molecules (washing stage), causing the cytoplasmic layer to be directly exposed to the solvent, facilitating the dissolution of active components in the solvent. Subsequently, there is a reduction in the rate of extraction (diffusion stage), controlled by a diffusion mechanism of the solute trapped inside the plant tissue. Second-order extraction models have also been reported as representative for extraction processes with traditional and

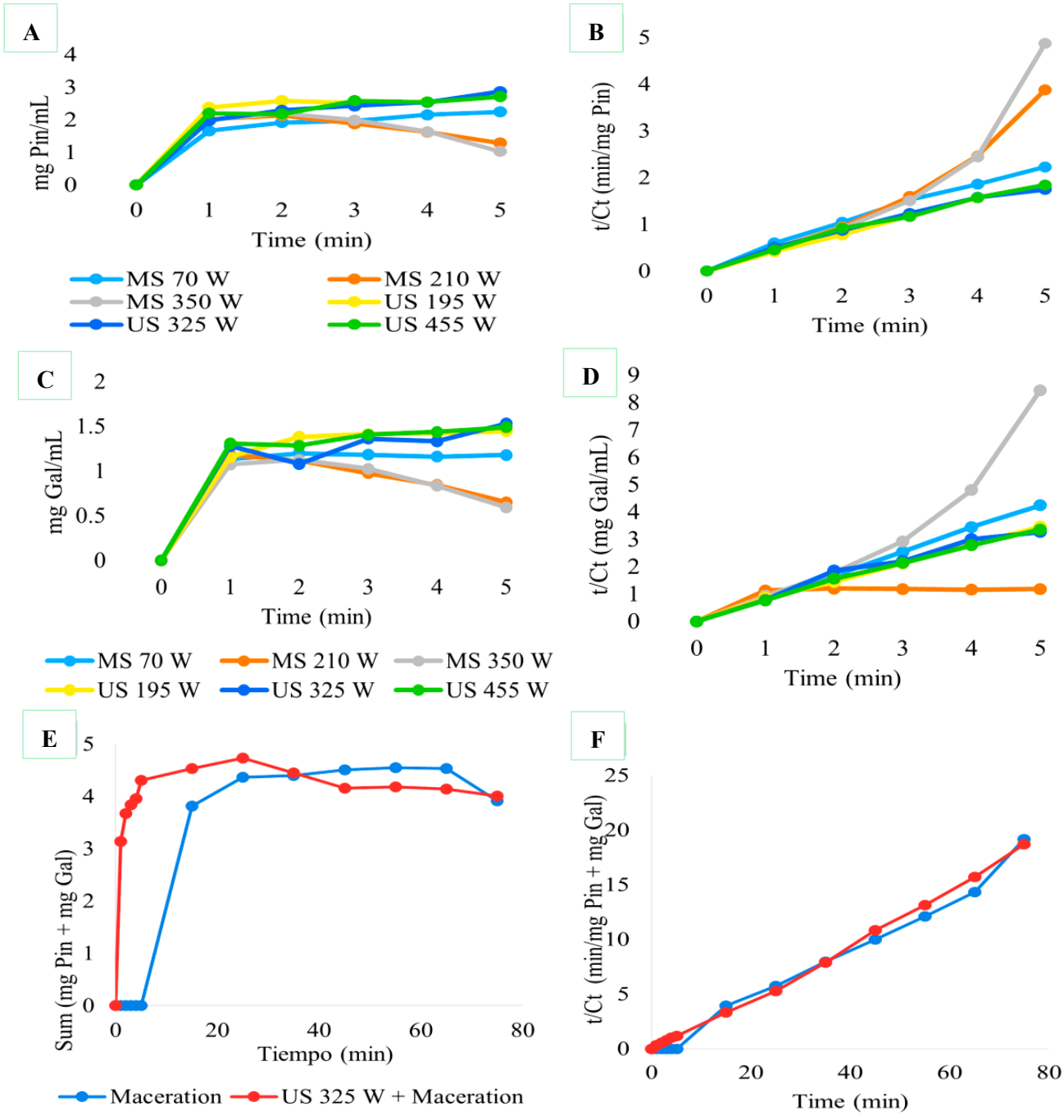

Figura 1. Evaluación de los pretratamientos para la extracción de pinocembrina y galangina del bagazo residual del orégano mexicano. US, ultrasonido. MS, microondas. **(A)** Cinéticas de extracción de pinocembrina (mg Pin/mL) en 5 m de pretratamiento. **(B)** Modelos cinéticos de segundo orden aplicado a las cinéticas correspondientes a pinocembrina. **(C)** Cinéticas de extracción de galangina (mg Gal/mL) en 5 m de pretratamiento. **(D)** Modelos cinéticos de segundo orden aplicado a las cinéticas correspondientes a galangina. **(E)** Cinéticas de extracción de pinocembrina y galangina, expresados en sumatoria (mg Pin + mg Gal/mL), obtenidas con maceración y con pretratamiento ultrasónico a 325 W durante 5 m más maceración. **(F)** Modelos cinéticos de segundo orden aplicado a las cinéticas de extracción correspondientes a la sumatoria de pinocembrina y galangina.

Figure 1. Evaluation of pretreatments for the extraction of pinocembrin and galangin from residual bagasse of Mexican oregano. US, ultrasound. MS, microwave. (**A**) Extraction kinetics of pinocembrin (mg Pin/mL) after 5 m of pretreatment. (**B**) Second-order kinetic models applied to the kinetics corresponding to pinocembrin. (**C**) Extraction kinetics of galangin (mg Gal/mL) after 5 m of pretreatment. (**D**) Second-order kinetic models applied to the kinetics corresponding to galangin. (**E**) Extraction kinetics of pinocembrin and galangin, expressed as a sum (mg Pin + mg Gal/mL), obtained with maceration and with ultrasonic pretreatment at 325 W for 5 m plus maceration. (**F**) Second-order kinetic models applied to the extraction kinetics corresponding to the summation of pinocembrin and galangin.

Tabla 2. Capacidad de extracción (Cs) de pinocembrina y galangina, constante de velocidad de extracción de segundo orden (k_2) y coeficiente de correlación (R^2), de los modelos cinéticos de pretratamientos (microondas y ultrasonido) a diferentes potencias de procesamiento, durante 5 minutos.

Table 2. Extraction capacity (Cs) of pinocembrin and galangin, second-order extraction rate constant (k_2) and correlation coefficient (R_2), of the kinetic models of pretreatments (microwaves and ultrasound) at different processing powers, for 5 minutes.

Pinocembrin			
Powers	**Cs (mg Pin/mL)**	**K_2 (1/mgPin*min)**	**R^2**
Microwave			
MS 70 W	2.164 ^{c}	2.310 ^{c}	0.990 *
MS 210 W	1.869 ^{d}	2.676 ^{b}	0.948 *
MS 350 W	1.651 ^{e}	3.028 ^{a}	0.868 **
Ultrasound			
US 195 W	2.652 ^{a}	1.885 ^{e}	0.995 *
US 325 W	2.677 ^{a}	1.908 ^{d}	0.981 *
US 455 W	2.620 ^{b}	1.868 ^{e}	0.990 *
Galangin			
Powers	**Cs (mg Gal/mL)**	**K_2 (1/mgGal*min)**	**R^2**
Microwave			
MS 70 W	1.468 ^{c}	3.406 ^{c}	0.999 *
MS 210 W	0.958 ^{d}	5.221 ^{b}	0.941 **
MS 350 W	0.908 ^{e}	5.504 ^{a}	0.908 ***
Ultrasound			
US 195 W	1.782 ^{b}	2.806 ^{e}	0.998 *
US 325 W	1.805 ^{a}	2.854 ^{d}	0.970 **
US 455 W	1.752 ^{b}	2.771 ^{f}	0.996 *

The different superscript letters and asterisks indicated that the Tukey test revealed significant differences between the samples ($p < 0.05$).

ultrasound-assisted methods for extracting Mexican oregano and pomegranate peel (Pan *et al*., 2012; Qu *et al*., 2010; Flores-Martínez *et al*., 2016).

The appropriate strength of an extraction pretreatment can facilitate the step of washing and, consequently, reduce the extraction time. For pinocembrin and galangin, the results of the second-order extraction rate constant (k_2) indicate that the extraction rate with microwave at 350 W is the highest, followed by microwave at 210 and 70 W, while ultrasound presents lower rates. However, the extraction capacity does not show the same behavior with respect to the rate constant, since the pretreatment with the highest concentrations of pinocembrin and galangin was ultrasound at 325 W, while the microwave pretreatments presented the lowest concentrations (Table 2), tending to a possible degradation process (Figure 1A and 1C).

The efficiency of innovative techniques, such as ultrasound and microwaves, is based on the intensity and power they use, causing a generation of internal pressure in the cells, this process in turn leads to cell rupture, thus releasing the metabolites of interest (Zhao *et al*., 2020).

In relation to the use of microwave, an improvement in extraction yields and a reduction in times have been demonstrated. However, in a study on the stability of flavonoids when exposed to microwave radiation (Biesaga, 2011), increasing power levels was found to promote degradation. The localized heating generated by this process can have a negative effect on thermosensitive compounds, causing overheating and undesired solvent evaporation, and therefore, it should be avoided as the stability of these compounds tends to decrease with the increase in extraction temperature (Cissé *et al*., 2012). This leads to an accelerated rate of compounds degradation, leading to poor yields, which is why it is important to approach the use of microwaves in extraction processes with caution.

An important characteristic related to ultrasound is the decrease in extraction time, since in some studies it has been reported to have no significant effect on extraction performance in contrast to traditional methods (Stanisavljevic *et al*., 2007; Velickovic *et al*., 2008), however, highlights its ability to significantly reduce said process times. Unlike microwave, ultrasound at a higher intensity or power range not only improves yields but also accelerates the extraction process, independently of the radiation mode (Pan *et al*., 2012). This behavior was clearly evidenced in the kinetics evaluated in the present investigation, where an increase in pinocembrin and galangin extraction was observed at high powers, particularly at 325 W. This behavior is detailed in Table 3, where the percentage of selectivity for each pretreatment is shown. In general terms, the flavonoids evaluated exhibit a more efficient extraction through the use of ultrasound. Furthermore, it is important to analyze the addition behavior of the two flavonoids, given that these compounds present similar biological properties already reported, they could have a synergistic effect in their application.

Tabla 3. Selectividad de extracción de pinocembrina, galangina y sumatoria de los pretratamientos evaluados.
Table 3. Selectivity of pinocembrin and galangin extraction, and sum of the evaluated pretreatments.

Tratments	% Selectivity Pin	% Selectivity Gal	% Selectivity Sum
MS 70 W	32.07	16.85	48.92
MS 210 W	18.40	9.31	27.71
MS 350 W	11.39	6.57	17.97
US 195 W	39.37	20.64	60.01
US 325 W	47.75	25.49	73.24
US 455 W	38.73	21.25	59.98

It is evident that higher yields of pinocembrin and galangin are obtained when using ultrasound, when considering the sum (Sum) of these compounds, selectivity percentages of 59.98 to 73.24 % were achieved. In contrast, microwave treatments presented considerably lower selectivity percentages, varying between 17.97 to 48.92 %, which is attributed to the decrease or degradation behavior observed at the end of the extraction kinetics (Figure 1A, 1C).

Evaluation of the extraction process with and without pretreatment

To evaluate the effect of pretreatment on the pinocembrin and galangin extraction process, the pretreatment that showed the highest extraction capacity (Cs) and percentage of selectivity was selected, corresponding to ultrasound at 325 W (Tables 2 and 3), applied for 5 m prior to the maceration process. The second-order model was applied to analyze the kinetic data of extraction of pinocembrin and galangin with and without pretreatment, expressing the values as a sum (mg Pin + mg Gal/mL) (Figure 1E, 1F and Table 4).

In the pinocembrin and galangin extraction model, values of the rate constant (k_2) of 4.201 1/mgPin+Gal*min were observed for the maceration without pretreatment, while for the extraction process with pretreatment a value of 4.516 1/mgPin*min was obtained, suggesting that the extraction rate increased significantly with the previous pretreatment. In addition, the extraction capacity (Cs) was also higher in this treatment (5.951 mg Pin+Gal/mL), compared to simple maceration, which was 5.536 mg Pin+Gal/mL. These results confirm that the application of ultrasound pretreatment allows obtaining higher yields, showing a significant difference ($p<0.05$) in the results, with an increase in the extraction capacity (Table 4).

Tabla 4. Capacidad de extracción (Cs) de pinocembrina y galangina, constante de velocidad de extracción de segundo orden (k_2) y coeficiente de correlación (R2), de los modelos cinéticos del proceso de maceración sin pretratamiento y con pretratamiento (US 325 W durante 5 m).
Table 4. Extraction capacity (Cs) of pinocembrin and galangin, second-order extraction rate constant (k_2) and correlation coefficient (R_2), of the kinetic models of the maceration process without pretreatment and with pretreatment (US 325 W for 5 m).

Extraction	Cs (mg Pin + Gal/mL)	K_2 (1/mgPin*min)	R^2
Maceration	5.536 [b]	4.201 [b]	0.989**
Maceration and ultrasound	5.951 [a]	4.516 [a]	0.997*

The different superscript letters and asterisks indicated that the Tukey test revealed significant differences between the samples ($p < 0.05$).

Figure 1E shows the extraction kinetics of the maceration process with and without pretreatment, where the two extraction stages (washing and diffusion) are observed. For both treatments, the first stage occurs up to minute 25, however, a higher extraction rate is noted in the maceration with pretreatment (US 325 W for 5 m). The diffusion stage then continues, which is more gradual and reaches equilibrium, with a slight degradation observed at the last point of the kinetics. Figure 1F shows the behavior of t/Ct as a function of time, represented linearly (Eq. 2), which represents the concentration of the analyzed flavonoids in relation to the extraction time. The data were fitted to the second-order model, with correlation coefficients (R^2) of 0.997 and 0.982 with and without pretreatment, respectively.

In kinetic terms, an adequate extraction pretreatment can improve the washing stage (Chen *et al*., 2011) and consequently, increase yields and decrease extraction times, as shown in Table 4, where there is an increase in the rate constant and extraction capacity with the ultrasound at 325 W-complemented treatment. In addition, this treatment also showed the highest extraction rate in the washing step (Figure 1E). This in turn leads to a reduction in processing time, since a more efficient and faster extraction process of pinocembrin and galangin could be completed in the 25th minute, which is where the highest peak of these flavonoids is shown, a time that corresponds to 5 mof ultrasound and 20 m of maceration, instead of 60 m of conventional extraction. This represents a saving in cost and extraction time of 59 %. Applying ultrasound was shown to significantly reduce processing time, saving about 10 to 12 m (Liao *et al*., 2021), while the present study achieved a reduction in processing time of up to 35 m.

Likewise, this improvement in extraction when applying ultrasound pretreatment is reflected in a significant increase in the percentage of selectivity and phenolic content (Figure

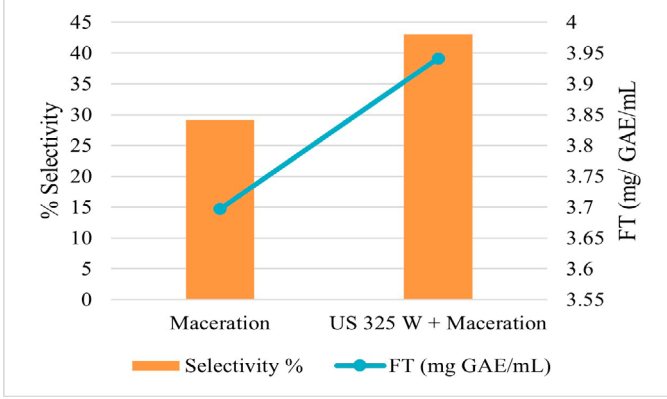

Figura 2. Porcentaje de selectividad (mg Pin + Gal/mg extracto seco) y contenido fenolico de los tratamientos de maceración y maceración con ultrasonido a 325 W.
Figure 2. Percentage of selectivity (mg Pin + Gal/mg dry extract) and phenolic content of the maceration and maceration with ultrasound at 325 W treatments.

2). The maceration treatment presented a selectivity percentage of 29 % and a phenolic content of 3.697 mg GAE/mL, while when applying the pretreatment, the selectivity increased significantly to 43 % and a phenolic content of 3.941 mg GAE/mL. These results suggest that pretreatment contributes positively to the extraction efficiency, improving the selectivity of pinocembrin and galangin and phenolic content. The mechanism of improvement observed through the use of ultrasound can be attributed to a mechanism that intensifies mass transfer due cavitation bubbles phenomenon in proximity to the cell walls, causing them changes in their permeability. As a consequence, a more effective contact is achieved between the solvent and the plant material, thus providing a more effective exposure of the active metabolite.

CONCLUSIONS

The present study has shown a first evaluation on extraction kinetics of flavonoids from oregano and its residual bagasse, applying pre-treatments with emerging technologies such as ultrasound and microwaves at different powers, prior to maceration. Second-order kinetic models were appropriate to represent the experimental results obtained, and these were useful to determine the statistical differences between treatments, seeking to improve yields and shorten processing times. The pretreatment that exhibited the highest extraction rate, selectivity and reduction in processing times was ultrasound at 325 W. When comparing the maceration process with and without pretreatment, it was observed that the applied second-order kinetic models could accurately predict the recovery of pinocembrin and galangin from the residual bagasse of Mexican oregano, adequately describing the experimental data in both treatments. Undoubtedly, the most effective treatment was the application of ultrasound as pretreatment, thus offering a highly effective option in the extraction processes of these flavonoids with a significant reduction in process times.

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CONFLICTS OF INTEREST

The authors declare there are no competing interests.

REFERENCES

Ali, M.C., Chen, J., Zhang, H., Li, Z., Zhao, L., and Qiu, H. 2019. Effective extraction of flavonoids from *Lycium barbarum L*. fruits by deep eutectic solvents-based ultrasound-assisted extraction. Talanta. 203: 16-22.

Arias, J., Mejía, J., Córdoba, Y., Martínez, J.R., Stashenko, E., and Valle, J.M. 2020. Optimization of flavonoids extraction from *Lippia graveolens* and *Lippia origanoides* chemotypes with ethanol-modified supercritical CO_2 after steam distillation. Industrial Crops and Products. 146: 112170.

Bajkacz, S., and Adamek, J. 2018. Development of a method based on natural deep eutectic solvents for extraction of flavonoids from food samples. Food Analytical Methods, 11: 1330-1344.

Biesaga, M. 2011. Influence of extraction methods on stability of flavonoids. Journal of Chromatography A. 18: 2505-2512.

Calvo-Irabién, L.M., Parra-Tabla, V., Acosta-Arriola, V., Escalante-Erosa, F., Díaz-Vera, L., Dziba, G.R., and Peña-Rodríjuez, L.M. 2014. Phytochemical diversity of the essential oils of Mexican oregano (*Lippia graveolens* Kunth) populations along an edapho-climatic gradient. Chemestry & Biodiversity. 11: 1010-21.

Chung, A.S., and Ferrara, N. 2011. Developmental and pathological angiogenesis. Annual Review of Cell and Developmental Biology. 27: 563-584.

Cid-Pérez, T.S., Ávila-Sosa, R., Ochoa-Velasco, C.E., Rivera-Chavira, B.E., and Nevárez-Moorillón, G. 2019. Antioxidant and antimicrobial activity of Mexican oregano (*Poliomintha longiflora*) essential oil, hydrosol and extracts from waste solid residues. Plants. 8: 22.

Cissé, M., Montet, D., Tapia, M.S., Loiseau, G., and Ducamp-Collin, M.N. 2012. Influence of temperature and relative humidity on the immobilized lactoperoxidase system in a functional chitosan film. Food Hydrocolloids. 28: 361-366.

Cortés-Chitala, M.C., Flores-Martínez, H., Orozco-Ávila, I., León-Campos, C., Suárez-Jacobo, Á., Estarrón-Espinosa, M., and López-Muraira, I. 2021. Identification and quantification of phenolic compounds from Mexican oregano (*Lippia graveolens* HBK) hydroethanolic extracts and evaluation of its antioxidant capacity. Molecules. 26: 702.

Codex Alimentarius. (2019). CODEX ALIMENTARIUS COMMISSION. Grupo de la producción y diseño editorial Servicio de Gestión de las Publicaciones FAO.

Cuevas-González, M.F., Flores-Martínez, H., León-Campos, C., López-Muraira, I.G., and Padilla-Rosa, J.D. 2021. Valorization of the residual bagasse of Mexican oregano (*Lippia graveolens* HBK) by obtaining fractions enriched in pinocembrin. Renewable Energy, Biomass & Sustainability. 3: 11-23.

Cheng, Y., Honggang, Z., Huiying, L., Lixin, L., Ruiqiu, S., Jingjing, G., Weihe, L., Min, Q., Shuai, S., Haiyan, B., Kai, H., Ruxia, D., Yiying, W., Yunyao, C., and Jiakun, B. 2019. Pinocembrin is in preparation for treating the application in pulmonary fibrosis disease drug. CN109806256A

Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A., and Abert-Vian, M. 2017. Review of green food processing techniques. Preservation, transformation, and extraction. IInnovative Food Science & Emerging Technologies. 41: 357-377.

Chung-Hung, C., Rozita, Y., and Gek-Cheng, N. 2014. Modeling and kinetics study of conventional and assisted batch solvent extraction. Chemical Engineering Research and Design. 92: 1169-1186.

Du, G., Ma, Y., Song, J., Li, L., Niu, Z. Wu, and S., Lv. 2018. Applications of pinocembrin in preparing anti-cerebral hemorrhage medicament. WO2018090358 (A1).

Flores-Martínez, H., León-Campos, C., Estarron-Espinosa, M., and Orozco-Avila, I. 2016. Process optimization for the extraction of antioxidants from Mexican oregano (*Lippia graveolens HBK*) by the response surface methodology (RSM) approach. Revista Mexicana de Ingeniería Química. 15: 773-785.

Guan, X., Li, Z., Zhu, S., Cheng, M., Ju, Y., Ren, Yang, G., L. and Min, D. 2021. Galangin attenuated cerebral ischemia-reperfusion injury by inhibition of ferroptosis through activating the SLC7A11/GPX4 axis in gerbils. Life Sciences. 264: 118660.

Mastelić, J., Jerković, I., Blažević, I., Poljak-Blaži, M., Borović, S., Ivančić-Baće, I., Smrečki, V., Žarković, N., Brčić-Kostic, K., Vikić-Topić, D., and Müller, N. 2018. Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives. Journal of Agricultural and Food Chemistry. 56: 3989-3996.

Martínez-Natarén, D.A., Parra-Tabla, V., Dzib, G., Acosta-Arriola, V., Canul-Puc, K.A., and Calvo-Irabién, L.M. 2012. Essential oil yield variation within and among wild populations of Mexican oregano (*Lippia graveolens* HBK- Verbenaceae), and its relation to climatic and edaphic conditions. Journal of Essential Oil Bearing Plants. 15: 589-601.

Michalaki, A., Karantonis, H.C., Kritikou, A.S., Thomaidis, N.S., and Dasenaki, M.E. 2023. Ultrasound-assisted extraction of total phenolic compounds and antioxidant activity evaluation from oregano (*Origanum vulgare* ssp. Hirtum) using response surface methodology and identification of specific phenolic compounds with HPLC-PDA and Q-TOF-MS/MS. Molecules (Basel, Switzerland). 28: 2033.

Morais, L.A.S. 2009. Influência dos fatores abióticos na composição química dos óleos essenciais. Horticultura Brasileira. 27: S4050-S4063.

Koksal, O., Gunes, E., Ozer, O.O., and Ozden, M. 2010. Analysis of effective factors on information sources at Turkish Oregano farms. African Journal of Agricultural Research. 5: 142-149.

Liao, J., Guo, Z., and Yu, G. 2021. Process intensification and kinetic studies of ultrasound-assisted extraction of flavonoids from peanut shells. Ultrasonics Sonochemistry. 76: 105661.

Lin, L.Z., Mukhopadhyay, S., Robbins, R.J., and Harnly, J.M. 2007. Identification and quantification of flavonoids of Mexican oregano (*Lippia graveolens*) by LC-DAD-ESI/MS analysis. Journal of food composition and analysis. 20: 361-369.

Oreopoulou, A., Goussias, G., Tsimogiannis, D., and Oreopoulou, V. 2020. Hydro-alcoholic extraction kinetics of phenolics from oregano: Optimization of the extraction parameters. Food and Bioproducts Processing. 123: 378-389.

Pan, Z., Qu, W., Ma, H., Atungulu, G.G., and McHugh T. 2012. Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. Ultrason Sonochem. 19: 365-72.

Psarrou, I., Oreopoulou, A., Tsimogiannis, D., and Oreopoulou, V. 2020 Extraction kinetics of phenolic antioxidants from the hydro distillation residues of rosemary and effect of pretreatment and extraction parameters. Molecules. 25: 4520.

Qu, W., Pan, Z. and Ma, H. 2010. Extraction modeling and activities of antioxidants from pomegranate marc. Journal of Food Engineering. 99: 16-23.

Santos, P.C., Oliveira, P.J.A., Santos, C.A., Oliveira, C.E.M., Arrigoni-Blank, M.F., Matos, A.T., Santos, D.A., Barreto, A.P., and Fitzgerald, B. 2016. Harvest time and geographical origin affect the essential oil of *Lippia gracilis* Schauer. Industrial Crops and Products. 79: 205-210.

Soto-Armenta, L.C., Sacramento-Rivero, J.C., Acereto-Escoffié, P.O., Peraza-González, E. E., Reyes-Sosa, C.F., and Rocha, J. 2017. Extraction yield of essential oil from *Lippia graveolens* leaves by steam distillation at laboratory and pilot scale. Taylor & Francis. 20: 610-621.

Tao, J., Shen, C., Sun, Y., Chen, W., and Yan, G. 2018. Neuroprotective effects of pinocembrin on ischemia/reperfusion-induced brain injury by inhibiting autophagy. Biomedicine & Pharmacotherapy. 106: 1003-1010.

Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., Neng, N.R., Nogueira, J., Saraiva, J.A., and Nunes, M. 2013. Chemical composition and bioactivity of different oregano *(Origanum vulgare)* extracts and essential oil. Journal of the Science of Food and Agriculture. 93: 2707-2714.

Zhao, C.-C., Kim, P.-H., and Eun, J.-B. 2020. Influence of high-intensity ultrasound application on the physicochemical properties, isoflavone composition, and antioxidant activity of tofu whey. Lebensmittel-Wissenschaft Und Technologie. Food Science and Technology. 117:108618.

Zhi-Ting, L., Ying, Z., Xiu-Juan, Z., Tong-Tong, Z., Jin-Shan, Z., and Xiao-Qiang, C. 2023. Optimization of ultrasound-assisted extraction of flavonoids from *Portulaca oleracea* L., the extraction kinetics and bioactivity of the extract. Journal of Applied Research on Medicinal and Aromatic Plants. 37: 100512.