Research Article

Mexican Sage (Salvia officinalis) Extraction Using Factorial Design and Its Effect on Chemical and Antibacterial Properties

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The essential oils (EOs) extracted by hydrodistillation (HYDRO) and steam distillation (SD) from Mexican Salvia officinalis L were analyzed for yield, chemical composition (GC-MS), particle morphology (SEM), antioxidant activity (ABTS), and antibacterial activity against Enterobacter agglomerans, Citrobacter freundii, Salmonella sp, E.coli, and Pseudomonas aeruginosa. The influence of the factors (method, quantity, and sample) was evaluated using a 2^3 full factorial design, Pareto chart, normal probability plot, main effects, and interaction plots in variance analysis on yield and antioxidant activity. The quantity, methods, sample, and the methods × sample and methods × quantity interactions were the most significant factors on yield (%). The sample, methods, and quantity × sample interaction were significant for antioxidant activity. EO yields were between 0.35 and 1.27 (% w/w), and the highest value was obtained by the HYDRO method using 50 g of whole sage leaves. The antioxidant activity values were in the range of 2.35 to 3.44 mg Trolox equivalent/g. Camphor, limonene, camphene, and caryophyllene were the main compounds identified. Micrographs of sage leaves showed relevant changes in the structure after extraction. The antibacterial activity was confirmed with the inhibition diameter and inhibition percentage of all bacteria, and P. aeruginosa was the most resistant bacteria. Finally, S. officinalis EO potentials can be considered an alternative natural preservative for the food and pharmaceutical industries.

1. Introduction

Essential oils (EOs) are called ethereal or volatile oil and they have been widely used in traditional medicine. These compounds are extracted from the various parts of the plants and mostly used as food flavors [1]. The EOs are complex mixtures of several low-molecular-weight and volatile compounds, such as isoprenoids, mainly mono- and sesquiterpenes, short-chain alcohols, aldehydes, and simple phenols. The EOs are produced and secreted by glandular trichomes, specialized secretory tissues diffused onto the surface of plant organs, particularly flowers and leaves [2]. Sage species are generally known for their multiple pharmacological effects, including antibacterial, antiviral, antioxidative, anti-inflammatory, antidiabetic, cardiovascular, antitumor, and anticancer [3]. These beneficial activities are positively related to phenolic compounds, such as phenolic diterpenoids (carnosic acid, carnosol, and rosmanol), phenolic acids (caffeic acid, rosmarinic acid, and ferrulic acid), and flavonoids (luteolin derivatives and apigenin derivatives) [4, 5].

Within the main components of sage essential oil are 1,8-cineole (64.3%), α-pinene (6.5%), and camphor (6.1%) [6]. Damyanova et al. [7] found that the main compounds of the Bulgaria sage essential oil are α-thujone (26.68%), (E)-β-caryophyllene (7.47%), 1,8-cineole (7.19%), α-humulene (6.11%), β-pinene (5.44%), and camphor (4.84%).

The antioxidant activity of sage EO makes them useful as natural preservatives in food, cosmetics, and pharmaceutical
2. Materials and Methods

2.1. Plant Material. The sage (S. officinalis) was grown in Atlixco, Puebla, Mexico, and the dried leaves were purchased from a local herbal shop (brand name “Sagrado Corazón de Jesús”).

2.2. Sample Preparation. The whole and ground dried leaves of sage were used for the essential oil extraction process. The pulverized sample was sieved (420 ± 25 μm) using a Keck Sieve Shaker kit (Cole Parmer, Vernon Hills, IL, USA) while the dried samples were packed into plastic bags, sealed under vacuum, protected from light, and stored at room temperature until use.

2.3. Essential Oil Extraction. Two processes (steam distillation and hydrodistillation), two quantities (25 and 50 g), and two presentations (whole and ground) of sage leaves were used. The obtention processes were conducted in a Clevenger-type distillation apparatus. Moreover, the extraction time for each experiment was 120 min and the temperature until use.

2.4. Yield. This parameter was calculated by the following equation:

\[
\% \text{yield} = \frac{\text{mass of essential oil (g)}}{\text{mass of dry leaves (g)}} \times 100.
\]  

The essential oil with the highest yield was chosen, to carry out the chemical composition and antimicrobial activity.

2.5. Antioxidant Activity. The antioxidant activity was analyzed by the ABTS (2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonate) (Sigma-Aldrich, St. Louis, USA) method [13]. The ABTS** radical was obtained by reacting the ABTS (7 mM) with potassium persulfate (2.45 mM) (Sigma-Aldrich, St. Louis, USA) for 16 h at room temperature. Once the radical ABTS** was formed, it was diluted with ethanol to obtaining an initial absorbance (Ai) of \(0.7 \pm 0.02\), measured at 754 nm (Thermo Scientific™ GENESYS™ 20, Visible Spectrophotometer). The antioxidant capacity was measured by placing 3920 μL of the ABTS** radical-ethanol solution in a quartz spectrophotometer cell and 80 μL of essential oil (dissolved in ethanol, used as blank), thoroughly mixed, allowed to react for 7 min, and the final absorbance was measured (Af). To calculate the antioxidant activity, Trolox (T) (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used in concentrations from 0 to 0.2 mg/mL. The standard curve was inhibition (% = \(387.13\ (%/\text{mg Trolox/mL}) \times C\ (\text{mg Trolox/mL}) + 3.256\% (R^2 = 0.996)\), and the antioxidant activity in oils was expressed as mg Trolox equivalent/g essential oil (mg TE/g EO).

2.6. Compound Identification. The volatile chemical compounds were characterized by gas chromatography/mass spectrometry (GC-MS). A gas chromatograph equipped with a 5975 quadrupole mass selective detector (Agilent Technologies 6850N GC, Santa Clara, CA, USA) and an HP5-MS column (30 m in length and 0.25 mm in diameter) was required. Conditions for analysis were as follows: helium as carrier gas at a flow rate of 15.5 mL/min, an injector temperature of 250°C, an injection volume of 1 μL, a split ratio of 10:1, and programmed temperature starting at 60°C and increasing at 4°C/min until reaching 250°C. The ionization energy was 70 eV. The scanning mass range was 43–350 m/z. Identification of the volatile compounds was performed by comparing their mass spectra with mass spectra of the NIST (National Institute of Standards and Technology) database library and information published in the literature [14].

2.7. Scanning Electron Microscopy (SEM). The morphology of samples exposed to the hydrodistillation process was examined using a JEOL JSM-6610LV scanning electron microscope (Tokyo, Japan). A probe, attached to the microscope, was used to perform the chemical analysis by the dispersive X-ray energy technique. All samples were examined under a high vacuum and acceleration voltage of 20 kV.

2.8. Antibacterial Activity. The antibacterial properties were ascertained in triplicate against Enterobacter agglomerans, Citrobacter freundii, Salmonella sp, E. coli, and Pseudomonas aeruginosa. Gram-negative bacteria were isolated from foods and identified using their colonial morphology on selective media (MacConkey, Brilliant Green agar, and Cetrimide agar), Gram staining, and biochemical tests (IMViC). Automated system VITEK (bioMerieux, Mexico) and 16S
rRNA gene partial sequencing were used to confirm the identification of bacterial genera. The 16S rRNA partial gene sequences were compared to sequences deposited in the GenBank database of the National Center for Biotechnology Information (NCBI) by using the Basic Local Alignment Search Tool (BLAST) algorithm. The bacterial strains were stored in Trypticase Soy Broth (TSB) with 20% glycerol at −80°C. The bacteria were cultivated in TSB at optimal growth conditions overnight (24 h at 37°C), and an aliquot was again transferred to TSB and grown at 37°C with stirring. Suspensions containing approximately 10^7 CFU/mL were used for antimicrobial activity assays.

In vitro antibacterial activity of the essential oil of sage was evaluated by cup-plate method, and a cup of 9 mm diameter was made using a sterile cork borer in the center of the inoculated plate of Trypticase Soy Agar. 30 μL of sage extract concentrations (0, 25, 50, 75, and 100%) was added to each cup. They were agitated with a stir and PBS (saline buffer) was prepared. The inoculated plates were incubated for 24 h at 37°C [15].

After incubation, the mean inhibition zone diameter for each concentration was measured in millimeters, and all the studies were performed in triplicate. The blank cup containing 30 μL of PBS was used as a negative control. The results were expressed as inhibition diameters (mm) and the percentage of inhibition halo calculated according to the following equation:

\[
\text{inhibitory(%) = } \frac{D - \text{AD}}{\text{AD}} \times 100,
\]

where \(D\) corresponds to the diameter of the inhibition halo of the microbial agent exposed to different concentrations of the antimicrobial extract and \(\text{AD}\) was the diameter of the antibiotic inhibition halo (nalidixic acid, 45 mm).

2.9. Factorial Design of Experiments. The factorial design describes which factor shows more impact and influences over the other factors [16]. A 2^3 factorial design with three factors at two levels was employed (Table 1), and the factors and interaction effects, coefficients of the model, normal probability plot, and Pareto chart on essential oil yield (%) and antioxidant activity were analyzed. The order of the experiments was randomized to avoid systematic errors. The results were analyzed with the Minitab 18 software, and the main effects and interactions between factors were determined.

3. Results and Discussion

3.1. Yield. The coded values of variables with the responses (yield and antioxidant activity) are mentioned in Table 2. The lowest yield was 0.35% (w/w) when extracting 25 g of ground sage using the SD method. The highest (1.27%) was obtained by the HYDRO method using 50 g of the whole sample. The result obtained in this work is consistent with those reported by Baydar et al. [17] (1.43–3.24%), who used Clevenger-type apparatus for 3 h. Alike, Miguel et al. [6] obtained yields of sage oil of 2.0–2.1% (v/w) and the extraction was performed with the hydrodistillation method, and Russo et al. [18] found yields between 0.55 and 2.2% with 18 different types of sage essential oils of different collection sites in Italy, using hydrodistillation for 3 h. Kammoun El Euch et al. [19] reported, for the same method and time, a yield of 1.03% (w/w), to sage from Tunisia.

The use of whole sage leaves improved oil extraction compared to the powdered leaves; moreover, higher yields of essential oil were observed using 50 g of sample. Nonetheless, doubling the quantity of raw material did not result in double the yield. According to the full 2^3 factorial design, the main factors and interaction effects on yield are presented in Figure 1. Figure 1(a) shows the main effects of the methods, quantity, and sample on the yield (%). When the largest vertical line changed from level –1 to level +1, it shows the main variation on yield (%).

From the interaction plots (Figure 1(b)), the nonparallel lines between the lowest (–1) and highest (+1) level values imply that there was a two-way interaction with different slopes. The steeper line (highest gradient) and the largest difference between points indicated the main effect or interaction [20]. Also, these figures illustrate the interactions (positive and negative) between the methods, quantity, and sample regarding yield (%). The methods × quantity and methods × sample plots (Figure 1) describe a strong interaction between both factors. Regarding the methods × quantity interaction, the effect of quantity was significant at the low-level method. Likewise, the maximum % yield (1.27%) was achieved at the low-level method and high-level quantity. This value was higher than that reported for Algeria’s sage, processed by hydrodistillation (0.9% w/w) [21]. The sample effect was significant at the low-level method, which is shown in the methods × sample plot.

The maximum % yield was observed at the high-level sample with the low-level method and the high-level quantity. However, the quantity × sample plot describes a weaker interaction effect between the factors.

Therefore, the essential oil yield can be expressed with the following model with an adjusted square correlation coefficient \(R^2\) of 94.88%, fitting the statistical model quite well:

\[
\text{yield} = 0.1331 - 0.2798^*A + 0.01716^*B + 0.0333^*C + 0.00344^*A*B - 0.0511^A*C + 0.00200^B*C.
\]

The negative and positive signs of each coefficient were based on each independent variable effect on yield, while the magnitude of the coefficient denoted the degree of significance of each independent variable. The order of significance

| Table 1: Factors and levels used in the factorial design. |
|-----------------|-----------------|-----------------|
| Factor          | Low level (−1)  | High level (+1) |
| Method (A)      | HYDRO           | SD              |
| Quantity (B)    | 25 g            | 50 g            |
| Sample (C)      | Ground          | Whole           |


based on magnitude was in good agreement with the resulting sequence of the Pareto chart [22]. The ABC (methods, quantity, and sample) effect was insignificant when it was compared with other effects, so it was not included in the model equation.

The normal probability plot of the standardized effects ($P = 0.05$) showed the significance of each factor and their interactions on yield (Figure 2(a)). This plot can be separated into two regions: the region with above 50% indicated as positive coefficients (BC, AB, C, and B) and the region with below 50% indicated as negative coefficients (AC and A). Based on the Pareto chart (Figure 2(b)), the sequence of the significant terms and the main interaction effects with respect to the % yield had the following order: B (quantity) > A (method) > C (sample) > AC (method and sample) > AB (method and quantity) > BC (quantity and sample).

### 3.2. Antioxidant Activity

Table 2 shows the antioxidant activity of sage oils obtained by both extraction methods, and the highest activity ($3.44 \pm 0.17 \text{mg TE/g of EO}$) was observed with 25 g of ground leaf using the SD method. Conversely, the HYDRO method reported the lowest value ($2.35 \pm 0.05 \text{mg TE/g EO}$) using 25 g of whole leaf. These results are similar to those reported by Carnerio et al. [23], who mentioned the values of 14.46 and 36.00 mg Trolox/g in Eugenia klotzschiana Berg (Myrtaceae) oil, using hydrodistillation (2 h) and a modified Clevenger-type apparatus. Ribeiro-Santos et al. [24] analyzed the antioxidant activity of four commercial essential oils: Ocimum basilicum, Cinnamomum cassia, Cinnamomum zeylanicum, and Rosmarinus officinalis with results of 1.81, 4.17, 474.80, and 2.75 mg Trolox/g, respectively. Likewise, Conde-Hernández et al. [25, 26] evaluated rosemary (Rosmarinus officinalis) and pepper leaf (Piper auritum) essential oils obtained by steam distillation, and they reported antioxidant activity values of 2.7 mg Trolox/g and 1.8 mg Trolox/g, respectively. Our results about the antioxidant activity are consistent with previous studies, which have claimed that EO contains pigmented and hydrophilic antioxidant compounds. These oils reflect better activity with ABTS than DPPH radical. For this reason, the ABTS test results were chosen to be evaluated in this study. Moreover, the antioxidant efficiency of sage essential oils has been attributed mainly to its majoritarian components, such as monoterpenes and sesquiterpenes hydrocarbons (in this work, 65.5 and 5.58%, respectively).

This antioxidant activity is related to the complex mixture of essential oils, which are constituted by different mono- and sesquiterpenes, and they present synergistic effects between them [24, 27]. Therefore, the antioxidant activity results are a good indicative that sage essential oils could be a potential source of natural antioxidant foods.

### Table 2: Design matrix for yield and antioxidant activity.

| Runs | Methods (A) | Quantity (B) | Sample (C) | Yield (%) | Antioxidant activity (mg TE/g EO) |
|------|-------------|--------------|------------|-----------|----------------------------------|
| 1    | −1          | −1           | −1         | 0.61      | 3.21                             |
| 2    | 1           | −1           | −1         | 0.35      | 3.44                             |
| 3    | −1          | 1            | −1         | 0.93      | 3.11                             |
| 4    | 1           | 1            | −1         | 0.79      | 3.35                             |
| 5    | −1          | −1           | 1          | 0.90      | 2.35                             |
| 6    | 1           | −1           | 1          | 0.39      | 2.83                             |
| 7    | −1          | 1            | 1          | 1.27      | 2.61                             |
| 8    | 1           | 1            | 1          | 0.98      | 2.94                             |

**Figure 1:** Plots of (a) main effects and (b) interaction effects for yield (%).
According to the factorial design, Figure 3(a) shows the main effects of the three factors (methods, quantity, and sample) on antioxidant activity; specifically, the quantity main effect was not significant.

In another way, Figure 3(b) illustrates that the methods × quantity and methods × sample plots describe a weaker interaction effect between both factors on antioxidant activity. Based on the methods × quantity plot, the maximum antioxidant activity was observed at the high-level method (SD) and high-level quantity (50g). Meanwhile, the methods × sample plot indicates that the maximum antioxidant activity was achieved at the high-level method (SD) and low-level sample (ground). As well, the quantity × sample plot describes a stronger interaction effect between both factors.

The antioxidant activity is expressed with the next model (R² adj = 94.22%): specifically, the effect of ABC interaction (methods, quantity, and sample) was not significant; therefore, it was not included in the next equation:

\[
antioxidant \text{ activity} = 2.9095 + 0.2092 A + 0.00189 B - 0.5039 C - 0.00133 A B + 0.0433 A C + 0.00550 B C.
\]

The plot of normal probability (Figure 4(a)) shows the standardized effects on antioxidant activity: the regions with positive coefficients (BC and A) and negative coefficient (C). The factors and interactions surrounded with a circle were not significant (AC, B, and AB), while those are denoted with a square were significant.

According to the Pareto chart (Figure 4(b)), the sequence of the significant terms and main interaction effects on the antioxidant activity was as follows: C (sample) > A (method) > BC (quantity and sample) > AC (method and sample) > B (quantity) > AB (method and quantity). Finally, all main effects (single effects) and two-factor interactions were not aliased with any other main effect or two-factor interactions, which is reported similarly by Cacua et al. [28].

3.3. Analysis of Variance for Yield and Antioxidant Activity. The ANOVA results of yield (%) and antioxidant activity are shown in Tables 3 and 4, respectively. The sum of the squares used to estimate factors and Fisher’s F ratios (defined as the ratio of mean square effect and the mean square error) and P values (defined as the level of significance leading to the rejection of the null hypothesis) were also represented [29]. Table 3 shows that the studied factors (methods, quantity, and sample) and their 2-way interactions (methods × quantity and method × sample) were statistically significant on yield. While the factors (methods and sample) and their 2-way interactions (methods-sample and quantity-sample) were statistically significant for antioxidant activity (Table 4).

3.4. Compound Identification. In this study, nineteen compounds were identified by a percentage of areas, using gas chromatography-mass spectrometry, for the Mexican sage EO that presented the highest yield (HYDRO-50-W) (Table 5). Oxygen-containing monoterpenes (65.6%) constituted the majority fraction of the essential oils, with camphor (34.17%), limonene (13.73%), camphene (12.31%), and sabinene (5.43%).
and an oxygen-containing sesquiterpene, caryophyllene (5.58%). Camphor is being reported as the main compound in this work in similar percentages to what Kammoun El Euch et al. [19] reported.

Previous studies reported similar chemical composition for sage essential oil, α-thujone, 1,8-cineole, and camphor, however in different quantities [6, 7, 19, 30, 31]. Differences in the chemical composition of sage oil from different studies can be attributed to factors such as plant variety, country of origin, time of harvest, and climatic factors (such as climatic, seasonal, and geographical variations) [10, 25].

The compounds of *Salvia officinalis* for medical uses could be very promising for encouraging the population to consume these plants; besides, sage essential oils are substances generally recognized as safe.

3.5. Scanning Electron Microscopy (SEM). Micrographs of samples exposed to hydrodistillation using 25 and 50 g of whole leaves are shown in Figure 5. A smooth surface of the sample without treatment is shown. Sage structures treated with hydrodistillation show an irregular surface. Some holes

![Figure 3](image_url)  
**Figure 3:** Plots of (a) main effects and (b) interaction effects for antioxidant activity.

![Figure 4](image_url)  
**Figure 4:** (a) Normal probability plot of the standardized effects at $P = 0.05$ and (b) Pareto chart of the significance rank of main effects and interaction effects of extraction factors on antioxidant activity.
are also shown on the surface of sage. Most of the samples appear collapsed and ruptured. Such eruptions can facilitate the outpour of all jailed essential oil [32]. The extraction method resulted in clear physical changes in the structure.

3.6. Antibacterial Activity. The antimicrobial activity of *S. officinalis* essential oil was evaluated against five Gram-negative bacteria *Pseudomonas, Enterobacter, Citrobacter, Salmonella*, and *E. coli*. These bacteria were chosen as they are potentially pathogenic for humans and some of them are part of the group of total and fecal coliform. These microorganisms are often responsible for causing mild to severe illnesses that are transmitted via the fecal-oral route and have been detected in various foodstuffs and during food preparations [33].

The results show that *S. officinalis* essential oil has antimicrobial properties against studied microorganisms, and in all treatments, the inhibitory effect increased with the concentration of essential oil (Table 6). The diameters ranged from 11.25 to 24.25 mm corresponding to the genera *Enterobacter*. This same bacterium and *Citrobacter* showed higher sensitivity with inhibition percentages of 86.95% and 73.5%, respectively. According to the analyses carried out by Alizadeh and Shaabanin [3], bacteria that showed inhibition diameters less than 7 mm were considered resistant to

### Table 3: Analysis of variance (ANOVA) for antioxidant activity.

| Source            | Df | Sum of squares | Mean square | F value | P value |
|-------------------|----|----------------|-------------|---------|---------|
| Main effects      | 3  | 1.28795        | 0.429318    | 88.98   | ≤0.001  |
| Methods           | 1  | 0.36451        | 0.364514    | 75.55   | ≤0.001  |
| Quantity          | 1  | 0.73574        | 0.735735    | 152.49  | ≤0.001  |
| Sample            | 1  | 0.18771        | 0.187706    | 38.90   | ≤0.001  |
| 2-way interactions| 3  | 0.08126        | 0.027087    | 5.61    | 0.019   |
| Methods x Quantity| 1  | 0.02953        | 0.029532    | 6.12    | 0.035   |
| Methods x Sample  | 1  | 0.04172        | 0.041718    | 8.65    | 0.016   |
| Quantity x Sample | 1  | 0.01001        | 0.0100010   | 2.07    | 0.184   |
| Residual error    | 9  | 0.04342        | 0.004825    |         |         |
| Pure error        | 8  | 0.04049        | 0.005061    |         |         |
| Total             | 15 | 1.41264        |             |         |         |

### Table 4: Analysis of variance (ANOVA) for yield (%).

| Source            | Df | Sum of squares | Mean square | F value | P value |
|-------------------|----|----------------|-------------|---------|---------|
| Main effects      | 3  | 1.83252        | 0.61084     | 78.75   | ≤0.001  |
| Methods           | 1  | 0.40642        | 0.40642     | 52.40   | ≤0.001  |
| Quantity          | 1  | 0.00888        | 0.00888     | 1.15    | 0.312   |
| Sample            | 1  | 1.41722        | 1.41722     | 182.71  | ≤0.001  |
| 2-way interactions| 3  | 0.11004        | 0.03668     | 4.73    | 0.030   |
| Methods x Quantity| 1  | 0.00442        | 0.00442     | 0.57    | 0.470   |
| Methods x Sample  | 1  | 0.02999        | 0.02999     | 3.87    | 0.081   |
| Quantity x Sample | 1  | 0.07563        | 0.07563     | 9.75    | 0.012   |
| Residual error    | 9  | 0.06981        | 0.00776     |         |         |
| Pure error        | 8  | 0.06316        | 0.00789     |         |         |
| Total             | 15 | 2.01238        |             |         |         |

### Table 5: Compounds identified by GC-MS in HYDRO-50-W sage essential oil.

| No. | Chemical compounds       | Peak area (%) |
|-----|--------------------------|--------------|
| 1   | 1R-α-Pinene              | 2.25          |
| 2   | Camphene                 | 12.31         |
| 3   | β-Pinene                 | 1.81          |
| 4   | β-Myrcene                | 0.9           |
| 5   | Sabinene                 | 5.43          |
| 6   | Limonene                 | 13.73         |
| 7   | Terpinolene              | 1.94          |
| 8   | 2-Carene                 | 2.28          |
| 9   | Camphor                  | 34.17         |
| 10  | Borneol                  | 3.42          |

*Peak area (%) of essential oil components. Components with percentage ≥0.5% are presented.*

Journal of Chemistry 7
S. officinalis essential oil, while bacteria that showed diameters between 7 and 11 mm were dose-dependent and more than 11 mm were sensitive.

Even pathogens E. coli and Salmonella presented a similar sensitivity to 100% extracts. Regarding E. coli, inhibition percentages greater than 50% were observed with extracts of 50, 75, and 100% (Table 6), and these results coincide with those obtained by López de Ávila et al. [34] who analyzed the antimicrobial properties of sage with various pathogens of relevance in food safety through cup-plate and disk diffusion methods. Also, Pierozan et al. [35] evaluated the antimicrobial activity of the S. officinalis EO and obtained lower inhibition diameters than those attained in our study with values of 8 mm and 11 mm for E. coli and Salmonella, respectively. Ivanovic et al. [36] confirmed the activity against E. coli considering a MIC of 2,560 mg/mL. The differences between the methods to analyze antimicrobial activity depend on several factors such as the volatile nature of the components of the essential oils, which may evaporate during inoculation and incubation and the abilities of dispersion of the EO in the culture medium [37]. However, the cup-plate method is a common technique used for the evaluation of antibacterial ability due to its simplicity and as small amounts of sample are required [15].

P. aeruginosa showed no inhibition with the analyzed concentrations of the EO sage.

Table 6: Diameters and percentages of inhibition obtained with different concentrations of extracts from leaves of S. officinalis on different Gram-negative bacteria.

| Concent. Extract (%) | Diameter inhibition (mm) | Inhibition percentages (%) |
|----------------------|--------------------------|---------------------------|
| 25       | 50   | 75   | 100  | 25   | 50   | 75   | 100  |
| Enterobacter agglomerans | 11.75 ± 2.2 | 11.25 ± 1.3 | 16.0 ± 1.4 | 24.25 ± 0.8 | 42.13 ± 7.9 | 40.34 ± 4.5 | 57.37 ± 5.1 | 86.95 ± 2.0 |
| Citrobacter freundii   | 13.0 ± 3.7  | 16.25 ± 2.3 | 19.0 ± 1.2  | 20.5 ± 1.3  | 46.61 ± 1.0 | 58.26 ± 1.3 | 68.13 ± 4.1 | 73.5 ± 4.6  |
| E. coli               | 11.75 ± 2.9 | 14.75 ± 1.7 | 17.75 ± 0.9 | 19.25 ± 3.8 | 42.13 ± 1.7 | 52.88 ± 6.1 | 63.64 ± 3.6 | 69.0 ± 1.35 |
| Salmonella sp         | 11.50 ± 1.0 | 14.0 ± 0.82 | 15.50 ± 0.98 | 19.0 ± 0.14 | 41.23 ± 3.6 | 50.12 ± 2.9 | 55.58 ± 3.5 | 68.13 ± 5.2 |

P. aeruginosa did not show inhibition (diameter inhibition (mm) and inhibition percentages) with the analyzed concentrations of EO sage.
barrier which provides protection against the effects of toxic agents.

Studies on the essential oils of *Salvia officinalis* show that antimicrobial potency is generally correlated to the chemical composition of the oil [41]. Pierozan et al. [35] consider that the antimicrobial activity of *S. officinalis* could be attributed to components as α-thujone, 1,8-cineole, and camphor, and in this work, camphor is the main component of the essential oil (Table 5). Furthermore, it has been shown that the minority compounds of these EO can present a synergistic effect with the principal components.

The antimicrobial properties derived from *S. officinalis* are of great importance because they could be used as an alternative to the increasing resistance of traditional antibiotics against infections by pathogenic organisms [4].

### 4. Conclusions

In summary, these results demonstrate that the quantity, methods, sample, and the methods × sample and methods × quantity interactions were the most significant factors on yield (%). However, the sample, methods extraction, and quantity × sample interaction were significant for antioxidant activity. The antioxidant activity of oil obtained by ground sage with hydrodistillation and steam distillation was significantly equal. The main compounds found in sage oils were camphor, limonene, camphene, and Caryophyllene. Micrographs of the samples (obtained by SEM) undergone HYDRO indicated that the extraction method resulted in significant changes to the sage leaf structure. This founding can be useful for future research related to other plant extraction. Finally, the results obtained concluded that the sage EO inhibited the growth of *E. coli*, *C. freundii*, *E. agglomerans*, and *Salmonella* sp; however, *P. aeruginosa* was the most resistant bacteria.

### Data Availability

The data used to support the findings of this study are included within the article.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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