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OBTENCIÓN DE COMPUESTOS BIOACTIVOS DE *LAVANDULA Y ROSMARINUS OFFICINALIS*, EMPLEANDO EXTRACCIÓN CONVENCIONAL Y NO CONVENCIONAL

OBTANING BIOACTIVE COMPOUNDS FROM *LAVANDULA* AND *ROSMARINUS OFFICINALIS*, USING CONVENTIONAL AND NON-CONVENTIONAL EXTRACTION

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Resumen

Los compuestos bioactivos que se encuentran presentes en las plantas de *L. officinalis* y *R. officinalis*, se han usado ampliamente en diversas aplicaciones, debido a las propiedades que poseen, entre las que encuentran las antimicrobianas, anticancerígenas y antioxidantes. Existen diversas metodologías para extracción de estos compuestos, las cuales se clasifican en convencionales y no convencionales, sin embargo, algunas de ellas resultan ser ineficientes ya que presentan tiempos largos de extracción, altos costos y bajos porcentajes de rendimiento. En este trabajo de investigación se realizó la comparación de un método convencional y otro no convencional para extraer los compuestos bioactivos que se encuentran presentes en las hojas de *R. officinalis* y *L. officinalis*, utilizando como disolvente etanol y un tiempo de extracción de 25 minutos en ambas técnicas. La extracción por técnica no convencional es mucho más selectiva, debido a que en los bioensayos de antibiosis *in vitro* se obtuvieron halos de inhibición más amplios, que fueron de 17 mm para LAM y 16 mm en ROM, frente a *Staphylococcus aureus*. Además, mediante FTIR-ATR y HPLC-MS se observó que ambas metodologías permiten extraer compuestos de gran interés como los terpenos fenólicos, ácidos metoxicinámicos, ácidos hidroxicinámicos, catequinas y flavonoles. Concluyendo que estas metodologías son excelente alternativa para la extracción de compuestos bioactivos de *R. officinalis* y *L. officinalis*, puesto que se obtuvieron altos porcentajes de rendimiento y los extractos presentaron propiedades antibacterianas frente a *Escherichia coli* y *Staphylococcus aureus*.

Palabras clave: *Rosmarinus officinalis*, *Lavandula officinalis*, método convencional, método no convencional.

Abstract

The bioactive compounds present in *L. officinalis* and *R. officinalis* plants have been widely used in various applications, due to the properties they possess, including antimicrobial, anticancer and antioxidant activities. There are several methodologies for the extraction of these compounds, which are classified into conventional and non-conventional, however, some of them are inefficient because they have long extraction times, high costs and low yield percentages. Therefore, in this research work a comparison of a conventional and a non-conventional method to extract the bioactive compounds present in the leaves of *R. officinalis* and *L. officinalis* was carried out, using ethanol as an extracting solvent and an extraction time of 25 minutes in both techniques. Showing that the extraction by non-conventional technique is much more selective, due to the in vitro antibiosis bioassays, the largest inhibition halos were obtained, which were 17 mm for LAM and 16 mm in ROM, against *Staphylococcus aureus*. In addition, by means of FTIR-ATR and HPLC-MS it was observed that both methodologies allow the extraction of compounds of great interest such as phenolic terpenes, methoxycinnamic acid, hydroxycinnamic acid, catechins and flavonols. It was concluded that both methodologies are excellent alternatives for the extraction of bioactive compounds from *R. officinalis* and *L. officinalis*, since good yield percentages were obtained, and the extracts showed antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*.

Keywords: *Rosmarinus officinalis*, *Lavandula officinalis*, conventional method, non-conventional method.

Introduction

Plants are a very important source of bioactive compounds, which provide them with medicinal properties, such as antimicrobial, anticancer and antioxidant properties (Jain et al., 2019).

Lavender belongs to the Lamiaceae family in the Lavandula genus. It is a shrub that grows in Mediterranean and sunny areas, it can reach 20 to 80 cm in height. It has therapeutic effects and has been used since ancient times for the treatment of pathologies such as colds, diarrhea, fatigue, as well as respiratory problems; it is also used to moderate depression, as well as stress, anxiety and insomnia (Diass et al., 2023).

On the other hand, *Rosmarinus officinalis* is a medicinal plant that belongs to the Lamiaceae family and is commonly known as rosemary; it can reach up to 1.5 m in height. Several studies have shown that its leaves contain a high content of terpenes, catechins, phenolic terpenes, among which rosmarinic acid, carnosol and carnosic acid stand out (Andrade et al., 2018).

Both plants possess bioactive compounds such as polyphenols that have aroused great interest due to their antimicrobial, anticancer and antioxidant properties. Therefore, the extraction of these types of compounds is of great interest to the pharmaceutical, food and textile industries (Selvamuthukumaran et al., 2017).

The extraction methods traditionally used are the conventional ones, such as maceration, hydrodistillation, extraction by distillation, leaching, extraction with organic solvents and extraction with soxhlet, however, these techniques are not viable because they can degrade the secondary metabolites, long extraction times are used, they are of high costs and generally the most used solvents in these techniques are organic, volatile and toxic compounds (Wong et al., 2020).

Because of this, the search has begun for extraction alternatives that are more environmentally friendly by using non-toxic, less expensive solvents and shorter extraction times. These extraction methods are known as non-conventional, among which are microwave, ultrasound and plasma, which have been reported to have several advantages when compared with conventional methods, providing a considerable reduction in extraction time, energy savings, environmentally friendly solvents are used and also an increase in the percentage yield of secondary metabolites (Hernández et al., 2023).

Therefore, in this research work, a comparison is made between a conventional method (heating grill) and a non-conventional method (microwave) in the extraction of bioactive compounds from dried leaves of *Lavandula officinalis* and *Rosmarinus officinalis* using the same conditions in both techniques, with the objective of observing the differences in percentage yield, characterization and antibacterial tests.

Experimental

The leaves of *Rosmarinus officinalis* and *Lavandula officinalis* were left to dry at room temperature for seven days and finally crushed. Subsequently, ethanolic extracts were made by placing 100 g of organic matter in 1000 mL of ethanol, by conventional and non-conventional techniques.

The non-conventional extraction was carried out by microwave in a reactor (Ultrasonic Microwave Cooperative Workstation model XO-SM400) (Figure 1) and the conventional extraction in a heating grill, using a temperature of 70 ^{o}C and an extraction time of 25 minutes in both methods. After the time elapsed, they were rotaevaporated and the supernatant was left to dry at room temperature. Table 1, shows the abbreviations used for each of the extracts.

Table 1. Abbreviations of the extracts obtained.

Extract	Type of extraction
LAC	*Lavandula officinalis* obtained by conventional method (heating grill)
LAM	*Lavandula officinalis* obtained by non-conventional method (microwave)
ROC	*Rosmarinus officinalis* obtained by conventional method (heating grill)
ROM	*Rosmarinus officinalis* obtained by non-conventional method (microwave)

Figure 1. Microwave reactor (Ultrasonic Microwave Cooperative Workstation model XO-SM400).

Once the extracts were obtained, they were characterized by infrared spectroscopy (FTIR-ATR), using an IR Spectrum spectrophotometer, GX-Perkin-Elmer by the attenuated total reflection (ATR) technique and a diamond tip attachment.

For the high performance liquid chromatography-mass spectrometry (HPLC-MS) technique with a diode array detector (280 nm). Compound separation was performed on a Grace Denali c-18 column at 30 ºC. A Varían 500-MS ion trap, electrospray ionization (ESI), capillary voltage 90 V and mass acquisition range 100-2000 m/z were used.

In the thermogravimetric analysis, a TA instruments Q500 thermal analyzer was used, with a heating rate of 10 ºC / min and a temperature range of 30-700 ºC.

Finally, in the in vitro antibiosis bioassay, filter paper impregnated with dilutions of the extracts obtained were used and placed in nutrient agar against *Staphylococcus aureus* and *Escherichia coli*. The petri dishes were placed in an incubator for 24 hours at 37 ºC and finally the presence or absence of inhibition was observed.

Results and discussion

Different yield percentages were obtained in each of the methods used, as shown in Table 2, for *Rosmarinus officinalis* extracts yields of 14.11 % were obtained when using the non-conventional method and 13.89 % when using the conventional method, while for *Lavandula officinalis* extracts yield percentages of 13.25 % were obtained by the non-conventional method and 12.47 % when using the conventional method. As can be seen, the highest yield percentages were obtained when using the conventional method; however, the differences are not significant and in both methods the yield percentages are higher than those reported in the literature.

Lava et al. extracted bioactive compounds from the *Rosmarinus officinalis* plant by maceration for 72 hours and using ethanol as the extracting solvent, obtaining a yield of 5.48 % (Lava et al., 2022). Meanwhile, Campos et al. also obtained extracts of *Rosmarinus officinalis* by maceration, using a time of 15 days and ethanol as solvent, obtaining a yield percentage of 13.5 % (Campos et al., 2020). On the other hand, Flores et al. performed *Rosmarinus officinalis* extracts with ultrasound for 1 and 2 h and ethanol as solvent, where they obtained yield percentages of 11.10 % and 10.42 %, respectively (Flores et al., 2022).

Table 2. Percentage yields of Rosmarinus and Lavandula officinalis extracts.

Extract	Percentage yields (%)
ROC	13.89
ROM	14.11
LAC	12.47
LAM	13.25

Soler et al. carried out extractions of dried leaves of *Lavandula angustifolia* using a conventional and a non-conventional method. For the conventional method they used hydrodistillation, for which they weighed 130 g of plant matter and placed it in deionized water with times of 30, 60, 90 and 120 minutes, where they obtained yield percentages of 0.26, 0.35, 0.32, 0.22 % respectively. While for the conventional method they used ultrasound, placing 100 g of dry leaves in 80 mL of deionized water with times of 20, 25 and 27 minutes, who managed to obtain yield percentages of 0.31, 0.69 and 0.16 %, respectively. It was observed that the highest yield percentages were obtained when an ultrasound bath was used to obtain the extracts, which varied according to time (Soler et al., 2021).

The FTIR-ATR results of *Lavandula officinalis* showed similar behavior both in those obtained by the conventional and non-conventional methods, as shown in Figure 2 and 3 since the same bands are observed at 3394 cm^{-1} for the stretching of the O-H bond that refers to the presence of alcohols, phenols and ethers. At 2922 and 2851 cm^{-1} bands corresponding to methyl and methylene stretches are shown. The carbonyl (C=O) stretches are found at 1731 cm^{-1}, which is due to the presence of ketones and carboxylic acids.

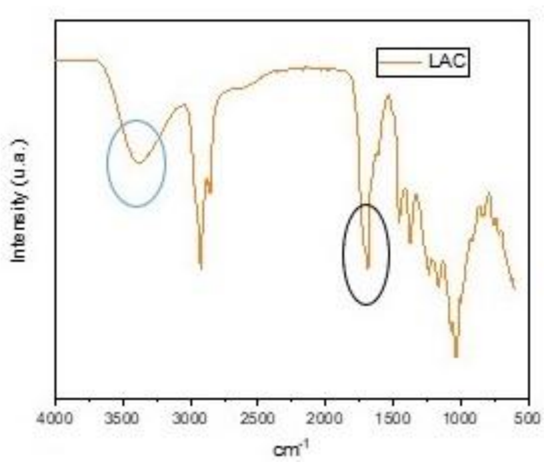

Figure 2. FTIR-ATR spectra of Lavandula officinalis extract obtained by heating grill.

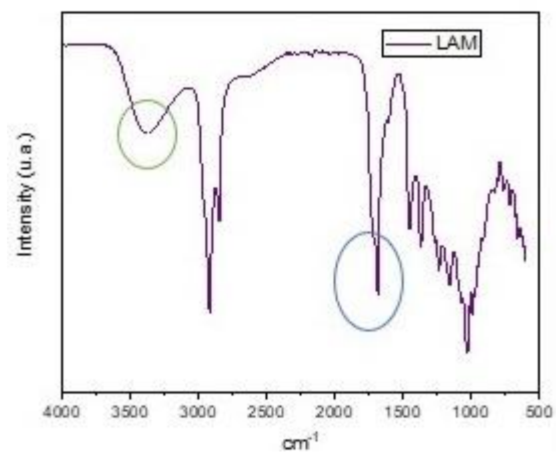

Figure 3. FTIR-ATR spectra of Lavandula officinalis extracts obtained by microwave.

On the other hand, in the extracts of *Rosmarinus officinalis*, the same behavior was also observed in both treatments (Figure 4 and 5). The O-H bond stretches are found at 3382 cm^{-1}, at 2928 and 2850 cm^{-1} methyl and methylene stretches are observed, while at 1701 cm^{-1} stretches of the C=O bond found in carboxylic acids and ketones are shown.

The FTIR-ATR results obtained agree with those published by Nidhi et al. who reported similar stretches in *Rosmarinus* and *Lavandula officinalis*. In *Lavandula officinalis* they highlighted the presence of C-H stretches at 2925 cm^{-1} and at 1748 cm^{-1} stretches of the C=O bond. While in *Rosmarinus officinalis*, they reported 2925 cm^{-1} bond stretches and at 1749 cm^{-1} C=O bond stretches (Nidhi et al., 2018; Silverstein et al., 2005).

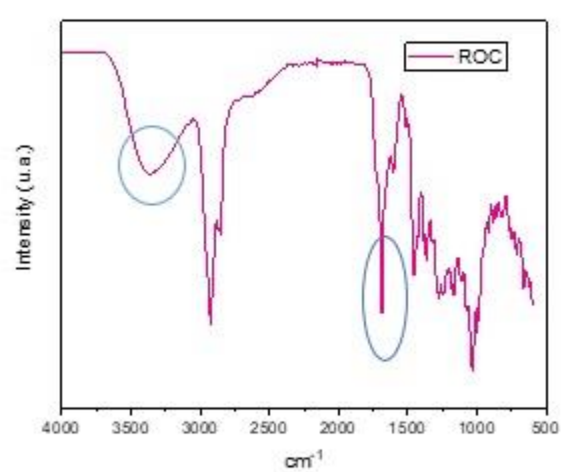

Figure 4. FTIR-ATR spectra of Rosmarinus officinalis extracts obtained by heating grill.

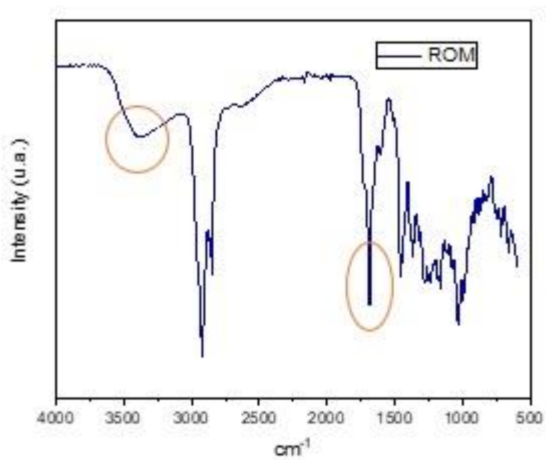

Figure 5. FTIR-ATR spectra of Rosmarinus officinalis extracts obtained by microwave.

The FITR-ATR results agree with those obtained in the HPLC-MS characterization, since the bands observed correspond to functional groups that are present in the chemical structures of the bioactive compounds of *Rosmarinus officinalis* (Figure 6) and *Lavandula officinalis* (Figure 7).

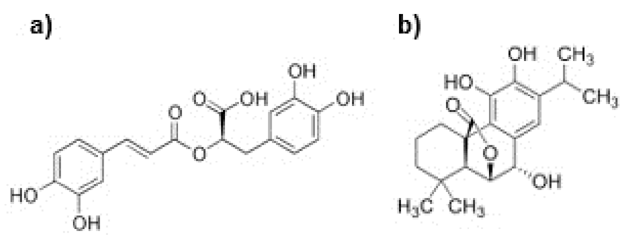

Figure 6. Chemical structures of bioactive compounds present in Rosmarinus officinalis leaves: a) rosmarinic acid and b) rosmanol.

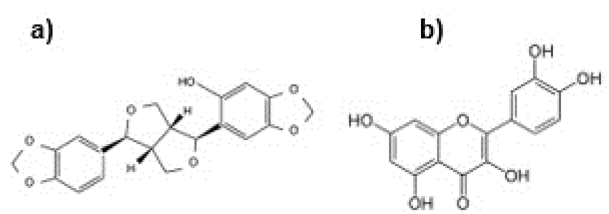

Figure 7. Chemical structures of bioactive compounds present in Lavandula officinalis leaves: a) sesaminol and b) quercetin.

The HPLC-MS analysis was able to identify families such as hydroxycinnamic acids, flavonols, methoxycinnamic acids, lignans and catechins. Table 3, shows the bioactive compounds extracted from *Lavandula officinalis* leaves, including sesaminol, medioresinol, quercetin, which have been reported in the literature as potent antimicrobials (Slighoua et al., 2022).

Table 3. HPLC-MS analysis of Lavandula officinalis extracts obtained by conventional and non-conventional methods.

Extract	TR*	Mass (m/z)	Compound	Family
LAC	1.257	352.7	1-Caffeoylquinic acid	Hydroxycinnamic acids
	5.793	341.0	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids
	23.06	325.0	Feruloyl tartaric acid	Methoxycinnamic acids
	30.624	354.9	Ferulic acid 4-O-glucoside	Methoxycinnamic acids
	33.572	387.0	Medioresinol	Lignans
	34.643	327.0	p-Coumaroyl tyrosine	Hydroxycinnamic acids
	43.276	300.8	Quercetin	Flavonols
	43.519	121.0	Benzoic acid	Hydroxybenzoic acids
	44.153	431.0	Apigenin 6-C-glucoside	Flavones
LAM	0.606	352.6	1-Caffeoylquinic acid	Hydroxycinnamic acids
	2.03	330.7	Galloyl glucose	Hydroxycinnamic acids
	2.84	368.6	Sesaminol	Lignans
	5.86	341.0	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids
	26.06	324.9	Feruloyl tartaric acid	Methoxycinnamic acids
	32.57	354.9	Ferulic acid 4-O-glucoside	Methoxycinnamic acids
	34.63	326.9	p-coumaroyl tyrosine	Hydroxycinnamic acids

**(TR) Retention time (min)*

Table 4, shows the bioactive compounds present in the leaves of *R. officinalis*, including rosmarinic acid and rosmanol, which are the main compounds to which the antibacterial properties of this plant are attributed (Alvarado et al., 2021).

Table 4. HPLC-MS analysis of Rosmarinus officinalis extracts obtained by conventional and non-conventional methods.

Muestra	TR*	Masa (m/z)	Compuesto	Familia
ROC	5.60	340.8	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids
	24.94	386.8	Medioresinol	Lignans
	26.26	304.8	(+)-Gallocatechin	Catechins
	32.77	476.8	Quercetin 3-O-glucuronide	Flavonols
	34.29	608.8	Quercetin 3-O-glucuronide	Flavonols
	35.96	358.7	Ácido rosmarínico	Methoxycinnamic acids
	38.83	652.7	Quercetin 3-O-(6"-acetyl-galactoside) 7-O-rhamnoside	Flavonols
	39.52	622.8	Apigenin 7-O-diglucuronide	Flavones
	42.93	324.9	Feruloyl tartaric acid	Methoxycinnamic acids
	45.76	344.8	Rosmanol	Phenolic terpenes
ROM	5.42	340.8	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids
	19.05	310.6	Caffeoyl tartaric acid	Hydroxycinnamic acids
	24.92	386.7	Medioresinol	Lignans
	26.43	304.8	(+)-Gallocatechin	Catechins
	32.42	476.8	Quercetin 3-O-glucuronide	Flavonols
	33.83	608.7	Quercetin 3-O-xylosyl-glucuronide	Flavonols
	35.43	358.7	Ácido rosmarínico	Hydroxycinnamic acids
	39.96	622.7	Apigenin 7-O-diglucuronide	Flavones
	43.17	324.8	Feruloyl tartaric acid	Methoxycinnamic acids

**(TR) Retention time (min)*

The major compound in the *Rosmarinus officinalis* plant was rosmarinic acid, which was found in the two extraction methods employed, with retention times of 35.96 (ROC) and 35.43 minutes (ROM) and a mass of 358. 7 (m/z), which agrees with that reported by Córdova et al, who reported the presence of rosmarinic acid in aqueous extracts of *Rosmarinus officinalis*, with a retention time of 13.21 minutes and a mass of 359 (m/z) (Córdova et al., 2021).

Figure 8 shows the thermogravimetric analysis of the ethanolic extracts of *Lavandula officinalis*, which, regardless of the treatment, show a gradual loss of 85 % in weight at approximately 316 ºC. Figure 9 shows the thermograms of the *Rosmarinus officinalis* extracts, showing the same behavior with a loss in weight of 85% at 318 ºC, which is attributed to the loss of water and some of the families present in the extracts. These temperatures are also an indication that the raw materials used are stable at high temperatures.

Flores et al. obtained ethanolic extracts of *Rosmarinus officinalis* obtained by ultrasound bath, indicating that in the thermogravimetric analysis they obtained a loss in weight of 50 % at a temperature of 350 ºC, demonstrating that the extracts are stable at temperatures above 300 ºC (Flores et al., 2022).

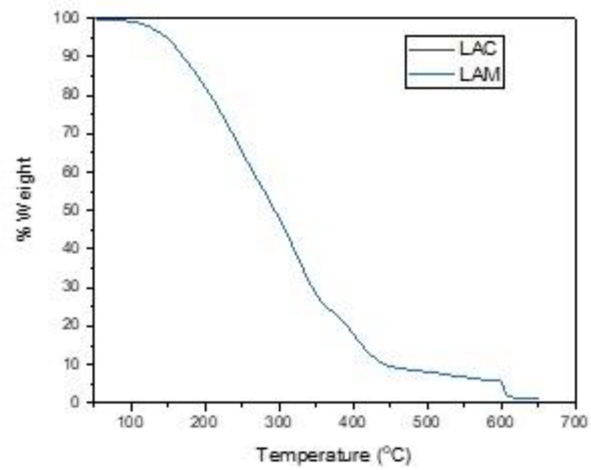

Figure 8. Thermograms of Lavandula officinalis extracts.

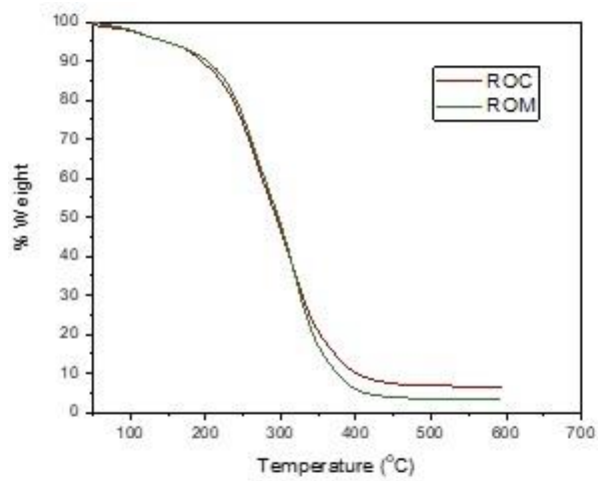

Figure 9. Thermograms of Rosmarinus officinalis extracts.

Finally, Figure 10 shows the analysis of the in vitro antibiosis bioassay. The largest inhibition halos were obtained with the extracts of *Rosmarinus* and *Lavandula officinalis* obtained by microwaves for both bacteria. Against *Staphylococcus aureus*, inhibition halos of 17 mm were obtained for LAM and 16 mm for

ROM, while for *Escherichia coli* they were 14 mm for LAM and 15 mm for ROM.

Escherichia coli is a gram-negative bacterium with a cell wall layer composed of lipopolysaccharides, a periplasmic space and a cytoplasmic membrane (Flores et al., 2022), unlike gram-positive bacteria such as *Staphylococcus aureus* whose cell wall contains peptidoglycan and teichoic acid (Hurtado et al., 2002; Cervantes et al., 2014). The bioactive compounds present in the extracts directly affect the cell membrane of bacteria and the cytotoxic activity directly affects the mitotic phase of gram-positive and gram-negative bacteria. Microorganisms such as *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* are susceptible to the bioactive compounds of *Lavandula* and *Rosmarinus officinalis* extracts, where rosmarinic acid, carnosol, flavonoids, caffeic acid and carnosic acid prevail (Pardo et al., 2022).

Montero et al. obtained an oily extract of *Rosmarinus officinalis* using steam distillation and evaluated the minimum inhibitory concentration against *Escherichia coli*, showing that there was no turbidity in the test and therefore there was no bacterial growth, concluding that the oily extract has antibacterial properties against this pathogen (Montero et al., 2017).

On the other hand, Fernandez et al. demonstrated that ethanolic extracts of *Rosmarinus officinalis* obtained by maceration have antibacterial properties against *Staphylococcus aureus*, since they reported inhibition halos of 26.88, 25.84 and 24.57 mm at concentrations of 100, 75 and 50% of the extract, respectively (Fernand ez et al., 2022).

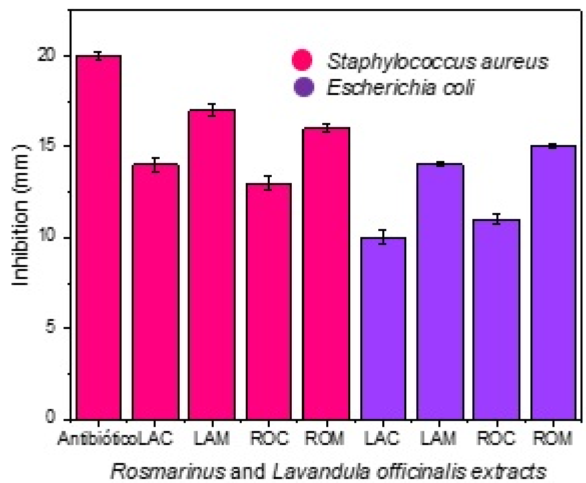

Figure 10. Determination of the antibacterial effect of ethanolic extracts of Rosmarinus and Lavandula officinalis against Staphylococcus aureus and Escherichia coli.

Conclusion

It is concluded that the leaves of *Lavandula* and *Rosmarinus officinalis* contain bioactive compounds that provide antibacterial properties. It was also demonstrated that both conventional and non-conventional methods are a good alternative for the extraction of bioactive compounds of interest, however, the microwave (non-conventional technique) presented better results in the in vitro antibiosis bioassay and it has also been demonstrated that it is a selective and greener extraction technique, which indicates that it is environmentally friendly.

Acknowledgments

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