Med Discoveries
An open access journal of science and medicine

Article Type: Research Article
Volume 4, Issue 4
Received: Mar 15, 2025
Accepted: Apr 07, 2025
Published Online: Apr 14, 2025

Antimicrobial and Antioxidant Activity of Crude Extracts of Medicinal Plants

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Abstract

The purpose of this research is to characterize the antibacterial and antioxidant activity of medicinal plant extracts and compare their biological activity with reported plant extracts. The study involved an *in vitro* evaluation of the antibacterial activity of the extracts against *Escherichia coli*, *Salmonella enteritidis*, and *Shigella dysenteriae*. Additionally, the antioxidant activity was determined using the DPPH method. Extracts from oregano, mint, thyme, lemongrass, sierrecilla, and tecomaxuchitl exhibited antibacterial activity at concentrations of 0.114, 0.456, and 0.912 µg/µL for *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella enteritidis*. For antioxidant activity, oregano (79.88%) and sierrecilla (77.96%) demonstrated high antioxidant potential.

Keywords: Antioxidant activity; Antibacterial activity; Plant extracts; Enterobacteria.

Introduction

Acute diarrheal diseases are a global public health issue and represent the third leading cause of death in children under the age of 5 [1]. While these diseases have various causes, the *Enterobacteriaceae* family is highly associated with these conditions.

This family is the largest and most heterogeneous group of Gram-Negative Bacilli (GNB) with clinical importance. They cause a wide range of diseases in humans, including gastrointestinal infections and opportunistic enterobacteria, such as *Salmonella enterica*, *Shigella spp.*, *Yersinia spp.*, and certain strains of *Escherichia coli* [2].

Various studies have highlighted the high prevalence of these bacteria and their resistance to different antimicrobials. This has prompted the search for alternative treatments, turning attention to medicinal plants.

Since ancient times, plants have been an essential source for alleviating diseases. In Mexico, the use of medicinal plants is deeply ingrained in its culture. Recently, this practice has regained importance in the treatment and prevention of diseases [3,4].

Materials and methods

The leaves of eight medicinal plants (*oregano, mint, thyme, lemon, lemongrass,* the bark of *cuachalalate, tecomaxuchitl,* and *sierrecilla*) were collected in the community of Atenxoxola in the municipality of Chilapa de Álvarez and in the municipal seat of San Luis Acatlán in the state of Guerrero, Mexico, except for *sierrecilla,* which was collected in the municipality of Axutla in the state of Puebla. After collection, the material was transported to the Laboratory of Integral Food and Natural Product Production to carry out preliminary tests at UAGro. For the selection of plant material, and showing spots, burns, or tissue damage caused by fungi or bacteria were discarded.

Sample processing

The material was washed with distilled water and dried at room temperature on racks to facilitate ventilation, ensuring proper drying and avoiding material loss.

Citation: Vargas-Álvarez D, Mendoza-Sabino G, Damián-Nava A, Rodríguez-Bataz E, Reyes-Ríos R, et al. Antimicrobial and Antioxidant Activity of Crude Extracts of Medicinal Plants. Med Discoveries. 2025; 4(4): 1251.

Determination of antibacterial activity

The antibacterial activity of the plant extracts was evaluated using the Kirby-Bauer method against the strains: *Salmonella enteritidis, Shigella dysenteriae,* and *Escherichia coli* provided by the Microbiology Laboratory of the Faculty of Chemical and Biological Sciences, UAGro.

Subsequently, bacterial suspensions were prepared in a 5% saline solution with equivalent turbidity.

500 mL of Müller-Hinton agar was prepared in Petri dishes. The bacteria were seeded after adjusting the bacterial suspension to 0.5 on the McFarland scale. The dishes were then incubated at 35±2ºC for 24 h, and the effect was determined by measuring the inhibition halos of bacterial growth.

0.1140 g of each crude extract was resuspended in 2 mL of ethanol in test tubes, resulting in all extracts having a concentration of 0.057 mg/mL. Subsequently, sterile paper discs were prepared with 2, 8, and 16 µL, yielding 0.114, 0.456, and 0.912 µg/µL of crude extract per disc. The discs were dried in a Petri dish and then placed in the culture media. The positive control was a 30 µg gentamicin disc, and the negative control was a disc with 3 µL of distilled water.

Determination of antioxidant activity

The antioxidant activity of the medicinal plant extracts in this study was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method.

0.5 µL of each extract was diluted to 2 mL with DPPH, with three repetitions for each extract. All reactions were incubated for 2 hours at room temperature in light-protected test tubes, and absorbance was measured at 520 nm using a spectrophotometer.

The results were expressed as IC50 inhibitory concentration (in mg/mL), indicating the amount of substance in 1 mL of reaction required to reduce the initial DPPH concentration by 50%.

Results

Antibacterial activity

The results of the antibacterial activity of the extracts at concentrations of 0.114 µg/µL showed variability in the ability to inhibit bacterial growth for each extract. *Mint, thyme, lemongrass,* and *tecomaxuchitl* inhibited *Escherichia coli,* while *sierrecilla* inhibited *Shigella dysenteriae.* Some extracts showed minimal activity, while others showed no activity.

Regarding the antibacterial activity of the extracts at concentrations of 0.456 µg/µL, variability in bacterial growth inhibition was observed for each extract. *Oregano, thyme,* and *lemongrass* inhibited *Escherichia coli,* while *lemon* showed better inhibition for *Shigella dysenteriae.* For *Salmonella enteritidis,* only *mint* and *lemon* showed inhibition. Some extracts showed minimal activity, while others showed no inhibitory activity.

At concentrations of 0.912 µg/µL, variability in bacterial growth inhibition was also observed for each extract. *Thyme* inhibited *Escherichia coli,* while *oregano* and *lemon* showed better inhibition for *Shigella dysenteriae.* For *Salmonella enteritidis,* only *tecomaxuchitl* showed inhibition. Some extracts showed minimal activity, while others showed no inhibitory activity (Tables 1-3).

Table 1: Antibacterial activity of extracts at concentrations of 0.114, 0.456, and 0.912 µg/µL for *Escherichia coli*.

	Escherichia coli							
	Oregano	**Mint**	**Thyme**	**Lemongrass**	**Lemon**	**Cuachalalate**	**Sierrecilla**	**Tecomaxuchitl**
0.114 µg µL	9 mm		**12 mm**		NP	NP	10 mm	**13 mm**
0.456 µg µL	**13 mm**	**12 mm**	**14 mm**	**13 mm**	9 mm	NP	10 mm	NP
0.912 µg µL	10 mm	NP	**14 mm**	9 mm	8 mm	9 mm	8 mm	NP
G. 30 µg µL	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm

Table 2: Antibacterial activity of extracts at concentrations of 0.114, 0.456, and 0.912 µg/µL for *Shigella dysenteriae*.

	Shigella dysenteriae							
	Oregano	**Mint**	**Tomillo**	**Lemongrass**	**Lemon**	**Cuachalalate**	**Sierrecilla**	**Tecomaxuchitl**
0.114 µg µL	8 mm	NP	10 mm	NP	NP	NP	**13 mm**	10 mm
0.456 µg µL	NP	NP	11 mm	8 mm	20 mm	NP	10 mm	NP
0.912 µg µL	**12 mm**	9 mm	NP	11 mm	**12 mm**	9 mm	11 mm	NP
G. 30 µg µL	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm

Table 3: Antibacterial activity of extracts at concentrations of 0.114, 0.456, and 0.912 µg/µL for *Salmonella enteritidis.*

	Salmonella enteritidis							
	Oregano	**Mint**	**Thyme**	**Lemongrass**	**Lemon**	**Cuachalalate**	**Sierrecilla**	**Tecomaxuchitl**
0.114 µg µL	8 mm	9 mm	10 mm	10 mm	9 mm	NP	NP	11 mm
0.456 µg µL	12 mm	**13 mm**	9 mm	8 mm	**14 mm**	NP	8 mm	NP
0.912 µg µL	NP	8 mm	NP	9 mm	12 mm	9 mm	10 mm	**13 mm**
G. 30 µg µL	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm	20 mm

Antioxidant activity

For the variable inhibition percentage, the means, variances, and graphs are presented for each of the factors studied and for the treatments.

The means (variances) of the inhibition percentage for plant extracts with different solvents were: Ethanol 39.67 (657.87) and Methanol 31.51 (633.54).

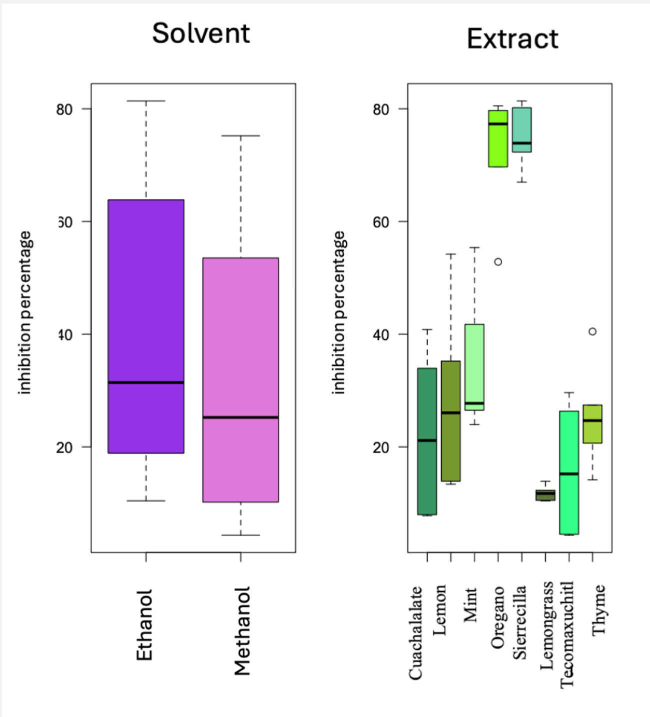

Figure 1: Distribution of inhibition percentage for solvents and extracts.

Discussion

Antibacterial activity

For antibacterial activity, the most notable inhibition at a concentration of 0.114 µg/µL is observed in *E. coli*, with the *tecomaxuchitl* extract showing a 13 mm inhibition halo, and the extracts of *mint, thyme,* and *lemongrass* each showing a 12 mm halo. No effect was observed for the *lemon* and *cuachalalate* extracts at this concentration. At a concentration of 0.456 µg/µL, the extracts with the highest activity were *thyme* (14 mm), *oregano,* and *lemongrass* (13 mm), while *cuachalalate* and *tecomaxuchitl* showed no activity. At a concentration of 0.912 µg/µL, the most prominent extract was *thyme* with a 14 mm inhibition halo, while *mint* and *tecomaxuchitl* showed no activity.

For *S. dysenteriae* at a concentration of 0.114 µg/µL, the most active extract was *sierrecilla* (13 mm), while *lemon, mint, lemongrass,* and *cuachalalate* showed no activity. At a concentration of 0.456 µg/µL, *lemon* showed high activity with a 20 mm inhibition halo, while *oregano, mint, cuachalalate,* and *tecomaxuchitl* showed no inhibition. At a concentration of 0.912 µg/µL, the most active extracts were *oregano* and *lemon* (12 mm), while *thyme* and *tecomaxuchitl* showed no activity.

For *S. enteritidis*, at the first concentration of 0.114 µg/µL, the most significant inhibition was observed with the *tecomaxuchitl* extract, while *cuachalalate* and *sierrecilla* showed no inhibition. At a concentration of 0.456 µg/µL, the inhibition was greatest for *lemon* (14 mm), *mint* (13 mm), and *oregano* (12 mm), while *cuachalalate* and *tecomaxuchitl* showed no activity. Finally, at a concentration of 0.912 µg/µL, the most prominent activity was observed with *tecomaxuchitl* (13 mm) and *lemon* (12 mm), while *oregano* and *thyme* showed no activity.

Studies conducted by Naik, MI (2010), Băicuș, A (2022), Afrin A (2023), demonstrate that *cuachalalate, oregano, thyme, mint, lemon,* and *lemongrass*, as reported by their respective authors, indeed exhibit antibacterial effects [5-7]. Based on the results, it is observed that the antibacterial activity of the extracts at different concentrations increases as the concentration of the extract increases. It should be noted that for *tecomaxuchitl* and *sierrecilla*, due to the lack of more concrete identification, our results cannot be refuted, as their uses are primarily empirical.

However, it is noteworthy that some extracts exhibit antimicrobial dose-dependent and the influence of the solvent on the extraction of metabolites required for antibacterial activity, therefore, the availability of crude plant extracts depends on the type of solvent used for extraction and the concentration of the plant material [8].

Antioxidant activity

Several studies have shown that oregano, mint and lemon have a good antioxidant capacity.

In the antioxidant activity, the inhibition percentage variable presents the means and variances for each of the factors studied and treatments, namely solvents (Ethanol and Methanol), extracts (*Oregano, Mint, Thyme, Lemongrass, Lemon, Cuachalalate, Sierrecilla,* and *Tecomaxuchitl*), and Solvent-Extract combinations (EtOr, EtSi, MeSi, MeOr, MeLi, EtMe, EtCu, EtTo, EtTe, MeMe, MeTo, EtLi, MeTl, EtTl, MeCu, MeTe).

It is worth noting that for the distribution of the inhibition percentage, Ethanol has a mean of 39.67%, while Methanol has a mean of 31.51%. Based on this result, Ethanol was chosen for further use as it is industrially produced, commercially available, residual, and non-toxic to health. Thus, it was used for the development or resuspension of the extract and for carrying out antibacterial activity tests.

Regarding extract effectiveness, *oregano* and *sierrecilla* stand out. Although Ethanol was confirmed as the solvent to be used for the study, Methanol was also employed to compare its efficacy.

Conclusion

Extracts from oregano, mint, thyme, lemongrass, sierrecilla, and tecomaxuchitl exhibited antibacterial activity at several concentrations for *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella enteritidis*.

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