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Antimicrobial Effects of Ethanol Extracts from *Uncaria tomentosa*, *Haematoxylum brasiletto*, and *Cymbopogon citratus* on Multidrug-Resistant Clinical Strains

Efectos antimicrobianos de extractos etanólicos de *Uncaria tomentosa*, *Haematoxylum brasiletto* y *Cymbopogon citratus* sobre cepas clínicas multirresistentes a medicamentos

García-Hernández, D.G.¹, Martínez- Santoyo, J.A.¹, Rivas-Morales, C.¹, Sánchez-García, E.¹, Leos-Rivas, C.¹, Flores-Gutiérrez, F.M.², Méndez-López, L.F.², Heya, M.S.^{2*}

¹ Facultad de Ciencias Biológicas, Departamento de Química, Universidad Autónoma de Nuevo León, San Nicolás de los Garza 66455, Nuevo León, México; DGG-H (<https://orcid.org/0000-0001-8409-139X>).

² Facultad de Salud Pública y Nutrición, Universidad Autónoma de Nuevo León, Ave. Pedro de Alba S/N & Ave. Manuel L. Barragán, San Nicolás de los Garza 64460, Nuevo León, México; MSH (<https://orcid.org/0000-0002-6886-4724>), FMFG (<https://orcid.org/0009-0008-9620-4759>).

david.garciahrz@uanl.edu.mx; javier.martinezsa@uanl.edu.mx; catalinarivas@yahoo.com.mx; eduardo.sanchezgrc@uanl.edu.mx; catalinaleosrivas@yahoo.com; ffloresg@uanl.edu.mx; luis.mendezlop@uanl.edu.mx; michel.heyax@uanl.edu.mx*

Technological innovation: Pharmacological development of antibiotics based on medicinal plants.

Industry application area: This research focuses on the pharmacological effects of plant extracts as a novel alternative for the treatment of bacterial infections.

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Resumen

Antecedentes: La resistencia antimicrobiana es una de las principales amenazas para la salud y el desarrollo globales. Según la Organización Mundial de la Salud (OMS), la resistencia bacteriana fue directamente responsable de 1.27 millones de muertes en todo el mundo en 2019. Como resultado, la industria farmacéutica ha renovado su interés en los productos naturales y su potencial aplicación en la búsqueda de nuevos medicamentos más eficientes para combatir las enfermedades humanas. Este estudio tuvo como objetivo determinar la actividad antimicrobiana de tres extractos etanólicos de plantas: palo de Brasil, uña de gato y zacate limón, contra bacterias multirresistentes a medicamentos. **Métodos.** La actividad biológica se determinó según el protocolo M26-A del Instituto de Estándares de Laboratorio Clínico. **Resultados.** Se observó inhibición del crecimiento para *H. brasiletto* (50 mg/mL) contra bacterias Gram-positivas, como *S. aureus* ATCC BAA-44,

utilizando los métodos de difusión en placa y microdilución en pozos, con una concentración mínima inhibitoria de 6.25 mg/mL y un halo de inhibición de 31 mm de diámetro. De manera similar, *P. aeruginosa* 27853, *E. faecalis* 29212 y *A. baumannii* 27853 mostraron inhibición a una concentración de 12.5 mg/mL y 6.25 mg/mL respectivamente, y diámetros de inhibición de 12, 21 y 16 mm, respectivamente. *U. tomentosa* presentó actividad bacteriostática, reduciendo la población a unas pocas colonias típicas del patógeno a una concentración de 200 mg/mL. *C. citratus* no mostró actividad bactericida o bacteriostática contra las bacterias en estudio. Los antibiogramas realizados en cepas de referencia (ATCC) mostraron resistencia a la mayoría de los antibióticos, pero susceptibilidad al antibiótico Ciprofloxacina. **Conclusión:** Basándonos en los resultados obtenidos, se recomiendan los extractos de plantas medicinales como una alternativa para tratamientos y/o como adyuvantes para problemas infecciosos causados por cepas multirresistentes a medicamentos.

Palabras clave: Multirresistente; Extracto vegetal; potencial antibacteriano.

Abstract

Background: Antimicrobial resistance represents one of the most significant threats to global health and development. According to the World Health Organization (WHO), bacterial resistance was directly responsible for 1.27 million deaths worldwide in 2019. Consequently, the pharmaceutical industry has renewed its interest in natural products and their potential application in the search for more effective drugs to combat human diseases. This study aimed to determine the antimicrobial activity of three ethanolic plant extracts—Brazilwood (*Haematoxylum brasiletto*), cat's claw (*Uncaria tomentosa*), and lemongrass (*Cymbopogon citratus*)—against multidrug-resistant bacteria. **Methods:** Biological activity was determined following the M26-A protocol of the Clinical and Laboratory Standards Institute (CLSI). **Results:** Growth inhibition was observed for *H. brasiletto* (50 mg/mL) against Gram-positive bacteria, such as *S. aureus* ATCC BAA-44, using agar diffusion and microdilution methods, with a minimum inhibitory concentration (MIC) of 6.25 mg/mL and an inhibition zone of 31 mm in diameter. Similarly, *P. aeruginosa* 27853, *E. faecalis* 29212, and *A. baumannii* 27853 showed inhibition at concentrations of 12.5 mg/mL and 6.25 mg/mL, respectively, with inhibition zone diameters of 12 mm, 21 mm, and 16 mm, respectively. *U. tomentosa* exhibited bacteriostatic activity, reducing the bacterial population to a few typical pathogen colonies at a concentration of 200 mg/mL. *C. citratus* did not demonstrate bactericidal or bacteriostatic activity against the bacteria under study. Antimicrobial susceptibility testing performed on reference strains (ATCC) revealed resistance to most antibiotics but susceptibility to ciprofloxacin. **Conclusion:** Based on the results obtained, medicinal plant extracts are recommended as alternatives for treatment and/or as adjuvants for infectious diseases caused by multidrug-resistant strains.

Keywords: Multidrug-resistant, Plant extract, Antibacterial potential.

1. Introduction

Plant-based medicine has been a common practice for centuries, and its importance in the fight against antimicrobial resistance cannot be underestimated. Indeed, antimicrobial resistance is a global problem that affects millions of people every year, and it is estimated that if no measures are taken to address this problem, antimicrobial resistance could become the leading cause of death worldwide by 2050 (WHO, 2024). In this context, plant-based medicine represents a promising alternative. Plants have been used for centuries to treat a wide range of diseases and have demonstrated effectiveness against numerous pathogens, including bacteria, viruses, and fungi (Arip et al., 2022; García-Hernández et al., 2025). Additionally, plants offer the advantage of being a renewable and sustainable source of medicinal compounds, making them a more attractive option than synthetic drugs (Chaachouay & Zidane, 2024). A notable example of the effectiveness of plant-based medicine is the use of *Artemisia annua* in the treatment of malaria (Chan et al., 2020; Martini et al., 2020; Roesch et al., 2025; Sun et al., 2025; Xu et al., 2025). In fact, *A. annua* is now used in many countries to treat malaria and has been shown to be a safer and more effective option than synthetic drugs (Sankar Sangeetha & Saravanakumar Padmavathi, 2025; Vashisth & Mishra, 2025; Villarreal et al., 2024). Another example is the use of curcumin, a bioactive compound found in turmeric, to treat inflammation and infection, (including bacterial and viral infections) (Deng et al., 2025; Fu et al., 2021; Obrzut et al., 2025).

Furthermore, plant-based medicine may help prevent antimicrobial resistance. For example, studies have reported that administering extracts of plants such as Echinacea and *Salvia* can reduce antimicrobial resistance in bacteria such as *Escherichia coli*. (Arip et al., 2022; Vaou et

al., 2021). Therefore, plant-based medicine represents a promising approach to address antimicrobial resistance. Plants have been used for centuries to treat a variety of diseases and have been shown to be effective against a wide range of pathogens. Additionally, plants are a renewable and sustainable source of medicines, making them a more attractive option than synthetic drugs.

Accordingly, this work aimed to determine the antimicrobial potential of *U. tomentosa*, *H. brasiletto*, and *C. citratus*, on multidrug-resistant bacteria of interest to public health as described like potential verotoxin produced by *E. coli* O157-H7, the potential of *U. tomentosa* as a natural products source and the benefits to health by Lemmongrass (Marques et al., 2025; Tazi et al., 2024), about the ethanol as a solvent, we decide to use it due to its polarity and low toxicity (Semenova et al., 2024).

2. Materials and Methods

2.1. Extraction of Plant Extracts

The dried and ground plant material was commercially obtained from Pacalli (Monterrey, Nuevo León, Mexico): (1) scientific name: *Cymbopogon citratus*, common name: Zacate limón; (2) scientific name: *Uncaria tomentosa*, common name: Uña de gato; (3) scientific name: *Haematoxylum brasiletto*, common name: Palo de Brasil. Extraction was performed by maceration. For this purpose, 30 g of dried and ground plant material were placed in a 500 mL Erlenmeyer flask with absolute ethanol (CTR Scientifics) to completely cover the plant material for one week. Subsequently, the solvent was removed from the obtained extract using a rotary evaporator (Yamato model RE200). Each crude extract was completely dried in a drying oven at a temperature below 50 °C (BTC-9100, TERLAB, Zapopan, Mexico) to determine its yield in accordance with Equation 1. The

extracts were stored in clean containers until use (Verde-Star et al., 2016).

$$\% R = \frac{(100)(g \text{ extract})}{g \text{ plant}} \quad (\text{Eq. 1})$$

The *U. tomentosa* extract was commercially obtained from Pacalli. To determine its concentration, 1 mL of the extract was placed in three pre-weighed vials and dried. The concentration was then calculated using a gravimetric method and expressed in mg/mL.

2.2. Antimicrobial activity

The biological activity was determined based on the M26-A Methods for Determining Bactericidal Activity of Antimicrobial Agents: Approved Guideline from the Clinical and Laboratory Standards Institute, with certain modifications (M26A, 1999). The strains were obtained from the Dr. José Eleuterio González University Hospital (UANL). *Pseudomonas aeruginosa* ATCC 27853, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC BAA-44, *Acinetobacter baumannii* ATCC 15308, and *Enterococcus faecalis* ATCC 29212 were used, in addition to their clinical isolates.

2.2.1. Agar diffusion

For this test, 100 μL of a bacterial suspension (1×10^6 CFU) were placed on plates containing Müller-Hinton agar medium. Then, 6 mm-diameter wells were made in the medium and 20 μL of each extract were added at concentrations of 50, 25, 12.5, and

6.25 mg/mL. As a negative control, 20 μL of the solvent used to dissolve the extracts were used. The plates were incubated for 24 hours at 37 °C. Subsequently, the inhibition zones were measured with a ruler and expressed in mm for each extract.

2.2.2. Determination of the Minimum Inhibitory Concentration (MIC).

The extracts that showed the largest inhibition zone by the plate diffusion method were subjected to determination of the minimum inhibitory concentration (MIC). In a 96-well microplate, 100 μL of the bacterial suspension (1×10^6 CFU) were added, followed by 100 μL of each extract sample prepared as serial dilutions ranging from 50 to 0.048 mg/mL. The microplates were then incubated for 24 hours at 37 °C, including a negative control and a growth control. To determine the MIC of each extract, absorbance was read on an ELx800 microplate reader (BIO-TEK) at 600 nm. The lowest concentration at which the absorbance was equal to that of the blank was considered the MIC.

3. Results and discussion

The organic extraction of the different plants used in the present study was carried out by maceration using 30 g of each dried plant. According to the results obtained (Table 1), low extraction yields were obtained, i.e., 3.66% and 8.5% for *C. citratus* and *H. brasiletto*, respectively.

Table 1. Yields of ethanolic extracts from the plants under study.

Plants	Part used	Yield (w/w, %)
<i>U. tomentosa</i>	NA	NA
<i>C. citratus</i>	Aerial	8.5
<i>H. brasiletto</i>	Aerial	3.66

NA = not applicable

In this study, growth inhibition was primarily observed for *S. aureus* ATCC BAA-44, with MIC values of 6.25 mg/mL and a maximum

inhibition zone diameter of 31 mm for *H. brasiletto* (50 mg/mL) using the plate diffusion method. Inhibitory activity was also

observed in *P. aeruginosa* 27853, *E. faecalis* 29212, and *A. baumannii* 27853 strains, with an MIC of 3.125 mg/mL and inhibition zone diameters of 12 mm, 21 mm, and 16 mm, respectively.

The *H. brasiletto* extract showed the highest activity among the three extracts; therefore,

its MIC was determined (Table 2) and it effectively inhibited bacterial growth. These results are consistent with previous studies that have demonstrated the antimicrobial activity of *H. brasiletto* ethanolic extracts (Gómez-Aguilar & Beltrán-Rodríguez, 2025; Heredia et al., 2005; Yasunaka et al., 2005).

Table 2. Mean inhibition zone diameters of *U. tomentosa*, *C. citratus*, and *H. brasiletto* at different concentrations.

Strains	Concentrations of <i>U. tomentosa</i> (mg/mL)				Concentrations of <i>C. citratus</i> (mg/mL)				Concentrations of <i>H. brasiletto</i> (mg/mL)			
	200	100	50	25	200	100	50	25	200	100	50	25
<i>E. faecalis</i> 29212	12	8	7	0	0	0	0	0	21	13	10	0
<i>A. baumannii</i> 15-308	8	5	0	0	0	0	0	0	16	12	10	8
<i>S. aureus</i> BAA-44	8	5	0	0	0	0	0	0	31	25	21	18
<i>P. aeruginosa</i> 27853	10	0	0	0	0	0	0	0	12	9	4	0
<i>P. aeruginosa</i> *	17	12	8	2.33	0	0	0	0	13	7	0	0
<i>E. faecalis</i> *	0	0	0	0	0	0	0	0	20	16	12	10
<i>A. baumannii</i> *	9	0	0	0	0	0	0	0	16	10	8	0
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)*	15	8	0	0	0	0	0	0	22	19	15	11

n = 3; diameters are expressed in millimeters. *Clinical isolate.

Regarding *U. tomentosa*, bacteriostatic activity was observed, with inhibition zones in which the bacterial population was reduced to a few typical colonies of the pathogen at the highest tested concentration (200

mg/mL). These results are consistent with previous studies that have demonstrated the antimicrobial activity of the plant's methanolic extracts (Rojas et al., 2016). No activity was observed for *C. citratus*.

Table 3. Minimum inhibitory concentrations (MICs) of the extracts with the greatest inhibitory activity.

Plant	Strain ATCC	MIC (mg/mL)
<i>H. brasiletto</i>	<i>P. aeruginosa</i>	3.125
	<i>E. faecalis</i>	3.125
	<i>A. baumannii</i>	3.125
	<i>S. aureus</i>	6.25
n = 3		

In another study, 32 extracts from 22 Mexican medicinal plants (15 different families) were analyzed to determine their antibacterial activity against *E. coli* and *S. aureus*. All extracts showed greater activity against *Staphylococcus*; among them was

Brazilwood (González et al., 2022; Mora Saavedra, 2024).

The plant extract and the positive control (cephalexin) showed greater effectiveness against Gram-positive bacteria. Because the

agar diffusion test is a semi-quantitative analysis, the polarity and chemical characteristics of the botanical extraction with absolute ethanol may have influenced the diffusion of the test compounds (Alvis et al., 2012; Aouadi et al., 2024).

The agar diffusion test was initially used to evaluate the inhibitory activity of the plant extracts. This assay is easy to perform and is frequently used to detect the antimicrobial activity of natural products.

Table 4. Antibiogram inhibition results for *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *A. baumannii* ATCC 15-308, and *S. aureus* ATCC BAA-44.

Antibiotic	<i>P. aeruginosa</i> ATCC 27853	<i>E. faecalis</i> ATCC 29212	<i>A. baumannii</i> ATCC 15-308	<i>S. aureus</i> ATCC BAA-44
Ampicillin	R	R	R	R
Carbenicillin	R	R	R	R
Cephalosporin	R	R	R	R
Cefotaxime	R	R	R	R
Ciprofloxacin	++++	++++	++++	R
Cephalexin	R	+++	+++	++++
Nitrofurantoin	R	++++	R	R
Amikacin	+++	R	+++	+++
Gentamicin	++++	R	++	R
Netilmicin	++++	R	++++	R
Norfloxacin	++++	++++	++++	R
Sulfamethoxazole/ Trimethoprim	R	R	R	+

Inhibition: R = resistant; + = 1–4 mm; ++ = 5–6 mm; +++ = 7–10 mm; ++++ > 10 mm.

Regarding the antibiograms performed on ATCC strains, the strains showed resistance to most antibiotics; however, they were sensitive to ciprofloxacin, cephalexin, amikacin, and norfloxacin (Table 4).

The mechanism of action of these drugs includes inhibiting bacterial DNA replication by targeting DNA gyrase, as well as binding to the 30S ribosomal subunit to form a non-functional 70S initiation complex, thereby interfering with protein synthesis. (Gad et al., 2024; Khwaza et al., 2024; Nayeem et al., 2024)

4. Conclusions

The inhibitory activity of three ethanolic extracts (*U. tomentosa*, *H. brasiletto*, and *C. citratus*) was determined against *P.*

aeruginosa, *E. faecalis*, *A. baumannii*, and *S. aureus*, including both ATCC reference strains and clinical isolates. The results were discussed based on inhibitory capacity, with *H. brasiletto* showing the highest efficacy and strong bactericidal activity against Gram-positive bacteria; activity was also observed against Gram-negative bacteria across the assays, with an MIC between 3.125 and 6.25 mg/mL. In contrast, *U. tomentosa* showed a bacteriostatic effect at 200 mg/mL, reducing the bacterial population around the wells. This concentration may have an adjuvant effect when combined with other antimicrobial drugs. Despite reports by other authors, no considerable inhibitory effect of *C. citratus* was observed under the conditions tested. For future work, it is recommended to study plants from the same family to compare

their phytochemical profiles and evaluate their antimicrobial capacity.

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