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Artículo de investigación

Antibacterial Activity of *Cnidoscolus urens* (L.) Arthur Ethanolic Leaf and Stem Extracts

Actividad antibacterial de extractos etanólicos de hojas y tallo de *Cnidoscolus urens* (L.) Arthur

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Abstract

This research explored the antibacterial activity of ethanolic extracts from the leaves and stems of *Cnidoscolus urens*L., a plant traditionally used in herbal medicine, against four different bacterial strains (*Bacillus sp, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli*). The study aimed to gauge the inhibitory effect of these extracts on selected bacteria and compare the antimicrobial activity of leaf and stem extracts. Using the agar diffusion technique with sensitivity discs, the antibacterial activity of the extracts was assessed. The major compounds in the extracts were identified using gas chromatography-mass spectrometry (GC/MS). The findings showed that both extracts significantly inhibited the growth of the evaluated bacteria, with the leaf extract demonstrating higher antimicrobial activity. Analysis of the extracts revealed differences in their chemical composition, including the presence of bioactive compounds such as n-Hexadecanoic acid and phytol. The study concludes that both leaf and stem extracts could be potential antibacterial agents, with the leaf extract showing greater activity, potentially due to its bioactive compounds. These results point to *C. urens* L. as a potential source of natural antimicrobial agents.

Keywords: Agar diffusion technique, antimicrobial activity, natural therapeutics, plant metabolites.

Resumen

Esta investigación exploró la actividad antibacteriana de los extractos etanólicos de hojas y tallos de *Cnidoscolus urens*L., una planta utilizada tradicionalmente en la medicina herbal, contra cuatro cepas bacterianas diferentes (*Bacillus sp, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli*). El estudio tuvo como objetivo evaluar el efecto inhibitorio de estos extractos sobre bacterias seleccionadas y comparar la actividad antimicrobiana de los extractos de hojas y tallos. Se empleó la técnica de difusión en agar con discos de sensibilidad para evaluar la actividad antibacteriana de los extractos. Los principales compuestos en los extractos fueron identificados mediante cromatografía de gases acoplada a espectrometría de masas (GC/MS). Los resultados mostraron que ambos extractos inhibieron significativamente el crecimiento de las bacterias evaluadas, con el extracto de hojas mostrando una mayor actividad antimicrobiana. El análisis de los extractos reveló diferencias en su composición química, incluyendo la presencia de compuestos bioactivos como ácido n-hexadecanoico y fitol. El estudio concluye que tanto los extractos de hojas como los de tallos podrían ser agentes antibacterianos potenciales, siendo el extracto de hojas el de mayor actividad, posiblemente debido a sus compuestos bioactivos. Estos hallazgos señalan a *C. urens* L. como una posible fuente de agentes antimicrobianos naturales.

Palabras Clave: Actividad antimicrobiana, técnica de difusión en agar, terapéuticos naturales, metabolitos vegetales.

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1 Introduction

The employment of medicinal plants in both the prevention and treatment of diverse diseases has been a practice deeply rooted in various cultures since antiquity [1]. Modern scientific research has revealed that the therapeutic properties of these plants predominantly derive from their secondary metabolites, demonstrating a broad spectrum of biological activities. *Cnidoscolus urens* L., previously classified as *Jatropha urens* L., has been recognized as one such plant with a wealth of medicinal properties, sparking interest within the scientific community [2, 3].

Previously classified as *Jatropha urens* L., *C. urens* (L.) Arthur is a plant native to tropical areas, flourishing at altitudes below 2100 meters above sea level. This genus is closely related to Manihot, with both belonging to the Manihoteae tribe of the Crotonoideae subfamily, within the Euphorbiaceae family. Despite its common grouping with *Jatropha, Cnidoscolus* is easily distinguishable due to its stinging hairs, glands, and the presence of a singular white floral envelope. In Latin America, *C. urens* is known by various common names, such as "mala mujer" in Mexico and "pringamosa" in Colombia. In Mexican folk medicine, it has been used to treat a wide array of ailments, including venereal diseases, back pain, stimulation of breast milk production, cholesterol reduction, and alcohol addiction treatment, among others [4].

C. urens L. is a species that shows great nutritional and medicinal potential. It is a xerophilic tree that can reach up to 6.0 meters in height. The plant has stinging trichomes on its leaves and stems, and it contains latex inside. The leaves of this plant are long, thick, lanceolate, and toothed, while others have lobed alternate palms. Additionally, the plant has milky latex and small flowers located at the tips of its dichotomously branched branches [3].

Different parts of the *C. urens* plant contain a diverse biochemical composition that includes anthocyanins, anthraquinones, coumarins, flavonoids, steroids, lignans, saponins, tannins, terpenoids, xanthines, and alkaloids [5]. The complexity of these chemical structures and their biosynthetic pathways distinguish these secondary metabolites. Recognized for their biological significance in herbal medicine, these metabolites exhibit a wide range of properties. Despite the vast diversity and high biotechnological potential identified in the *Cnidoscolus* genus, research on this species remains relatively unexplored. The existing literature primarily focuses on the characterization and identification of bioactive compounds and their potential biotechnological applications [3].

Presently, bacterial infections represent a growing challenge to human health due to escalating resistance to conventional antibiotics [6]. Consequently, there is an urgent requirement for new antibacterial agents that are not only effective against resistant bacteria but also have a reduced propensity for promoting resistance [7]. In this context, plant extracts and their secondary metabolites have demonstrated considerable potential for the development of novel therapeutic agents [8].

In this context, the present study probes the antibacterial activity of ethanol extracts from *C. urens* L. against both Gram-positive and Gram-negative bacteria. The extracts, prepared from lyophilized plant material, i.e., leaves and stems, were assessed using the agar diffusion technique [9] Given the known medicinal applications and biochemical composition of *C. urens* L., our study hypothesizes that its extracts could serve as potential antibacterial agents. Furthermore, we anticipate that the effectiveness of these extracts may vary based on the type of bacteria and the concentration of the administered extract.

2 Materials and methods

2.1 Extraction procedure

Cnidoscolus urens (L.) Arthur - Euphorbiaceae is preserved in the herbarium of the National University of Colombia under the code COL000390014. A total of 100 grams of each plant material (leaves and stems) were utilized in the study (Figure 1). The samples were collected on the outskirts of the city of Cúcuta, along the ring road, at coordinates 7°50'34"N 72°31'07"W, in late 2022. To preserve the plant's components and properties, the collected samples were lyophilized. This process enables the removal of water present in plant cells and tissues without altering the structure and chemical composition of the compounds, thereby resulting in completely dry plant material. Following lyophilization, 200 mL of ethanol (Merck, Germany) was added to each sample. These samples were then left in total darkness for 24 hours on a shaker (MAXQ 4450, Thermo Scientific TM, Marietta, United States) at 35 °C and 100 rpm. The resulting ethanol extracts were vacuum filtered using filter paper (Qual. Dia. 125mm, BOECO, Germany) and a vacuum pump (DOSIVAC, Buenos Aires, Argentina). These extracts were then concentrated under reduced pressure using a rotary evaporator (IKA®RV10, Wilmington, United States) at 50 rpm, 150 mbar, and 40 °C. The concentrated extracts were stored in amber vials at 4 °C for subsequent antibacterial analysis against Gram-positive bacteria (*Bacillus sp* and *Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*). The bacterial strains were obtained from the strain collection of the Universidad Francisco de Paula Santander (UFPS).

2.2 Identification of secondary metabolites by gas chromatography-mass spectrometry (GC-MS).

Chromatographic analysis was carried out on the ethanol extracts from *C. urens* (leaves and stems) using an AT6890 Series Plus gas chromatograph (Agilent Technologies, MSD 5973, Santa Clara, United States) in full scan mode. The analysis employed a DB-5MS column (5% phenyl-polymethylsiloxane, 60 m x 0.23 mm x 0.25 μm). Injection was conducted in Split mode (30:1) with the use of a Solid Phase Microextraction (SPME) device. Compound identification was facilitated by the Adams, Wiley, and NIST databases

2.3 Antimicrobial activity of *C. urens* against Gram-positive and Gram-negative bacteria.

The bacterial strains selected for this study include Gram-positive (*Staphylococcus aureus* and *Bacillus sp.*) and Gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria. These strains were chosen due to their clinical relevance and their documented ability to develop antimicrobial resistance. In particular, *S. aureus* and *K. pneumoniae* are among the

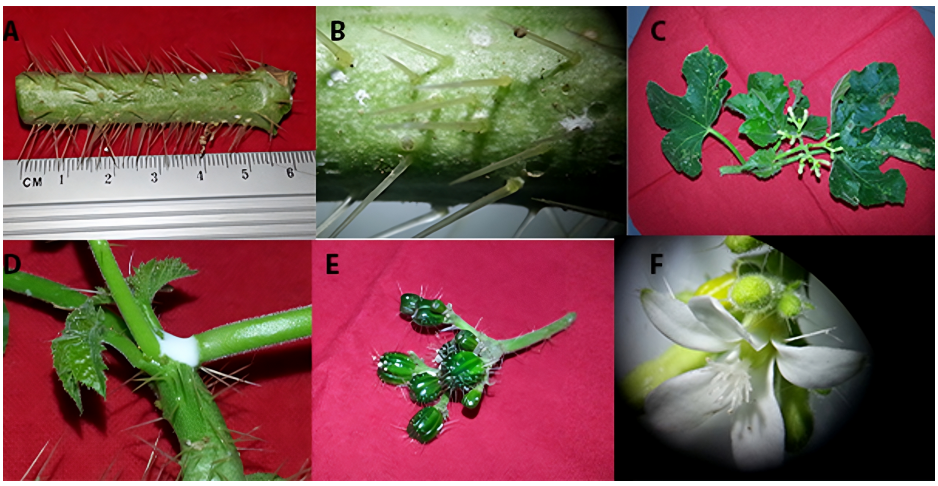

Figure 1: *Cnidoscolus urens* (L.) Arthur collected in Norte de Santander. A and B: trichomes; C: leaves; D: milky latex; E and F: inflorescences. Photographs by Chaves-Bedoya, previously published in Chaves-Bedoya and Ortiz-Rojas (2022), reused with authors' permission.

priority pathogens listed by the World Health Organization (WHO) because of their multidrug-resistant profiles [10]. E. coli is a commonly encountered enteric pathogen, also associated with antibiotic resistance. Bacillus sp., while less frequently resistant, serves as a representative Gram-positive environmental and opportunistic bacterium. The inclusion of both Gram-positive and Gram-negative bacteria allows for a broad evaluation of the antimicrobial potential of *C. urens* extracts.

The Kirby-Bauer method was utilized to assess the antimicrobial activity of the ethanol extracts from *C. urens* L. against Gram-positive bacteria (Bacillus sp and *Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*). The presence of inhibition zones quantitatively evaluated the antimicrobial activity of the ethanol extracts, with further statistical analysis conducted.

On nutrient agar plates, sterilized filter paper discs (sensidiscs) were impregnated with 25 $\mu\mu$L of each testing solution: the positive control (T1), a selective antibiotic for each bacterium; the negative control (T2), distilled water; the concentrated extract (T3); and dilutions 1:1 (T4), 1:2 (T5), 1:3 (T6), 1:4 (T7), and 1:5 (T8) of concentrated extract to ethanol. These agar plates were then incubated under specific conditions tailored for each bacterium for 24 hours. Subsequently, the diameters of the resulting inhibition zones were measured. For each assay, which was conducted in triplicate for statistical validation, a statistical analysis was performed using the Analysis of Variance (ANOVA) and Tukey's multiple comparison tests at an alpha level of 0.05.

3 Results and discussion

3.1 Major Compounds in the Ethanolic Extracts of *Cnidoscolus urens* L. Leaves and Stems

Among the major compounds identified by GC-MS in the ethanolic extract of *C. urens* L. leaves, fatty acids and terpenoids were observed. The following compounds were identified along with their retention times: n-Hexadecanoic acid (99%) at 37.117 min, 9,12-Octadecadienoic acid, ethyl ester (99%) at 43.2457 min, 9,12,15-Octadecatrienoic acid, methyl ester (91%) at 43.3918 min, and Phytol (76%) at 41.8446 min (Table 1).

Table 1: Major compounds identified in the ethanolic extract obtained from the bark of *C. urens* by Gas Chromatography-Mass Spectrometry (GC-MS). These compounds, potentially responsible for the observed antibacterial activity, reflect the chemical diversity and richness of this plant species.

TR (min)	Compounds	GC relative abundance
	Leaves	
23.5962	Diethyl phthalate	97%
33.9281	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R(1alpha,2beta,5alpha)]-	86%
35.0713	11-Tridecen-1-ol	38%
37.1170	n-Hexadecanoic acid	99%
37.8133	Nonadecanoic acid, ethyl ester	94%
41.8446	Phytol	76%
42.7213	Cyclooctene, 3-ethenyl-	91%
42.8159	Cyclodecanol	46%
43.2457	9,12-Octadecadienoic acid, ethyl ester	99%
43.3918	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	91%
43.5035	Ethyl oleate	99%
44.2943	Octadecanoic acid, ethyl ester	97%
	Stems	
23.7456	Diethyl phthalate	97%
37.0910	n-Hexadecanoic acid	97%
37.7786	Nonanoic acid, 9-bromo-, ethyl ester	83%
42.7555	3-Butyn-1-ol	9%
49.6577	Squalene	91%

Conversely, the major compounds identified by GC-MS in the ethanolic extract of *C. urens* stems revealed the presence of fatty acids and triterpenoids. The identified compounds included n-Hexadecanoic acid (97%) with a retention time of 37.091 min and Squalene with a retention time of 49.6577 min (Table 1).

3.2 Antimicrobial Activity of the Ethanolic Extracts of *C. urens* L.

In this study, the efficacy of ethanolic extracts from *C. urens* leaves and stems was evaluated in inhibiting the growth

of four distinct bacterial strains using the agar diffusion technique with sensitivity discs. Our results indicate that both extracts significantly inhibited bacterial growth, with differences in effectiveness depending on the bacterial strain and the applied treatment.

The antimicrobial activity of the extracts was tested against *Staphylococcus aureus, Bacillus sp., Klebsiella pneumoniae*, and *Escherichia coli*. The results showed that the leaf extract exhibited greater inhibition zones than the stem extract, particularly at higher dilutions (Figure 2). The inhibition varied among bacteria, with *S. aureus* being the most susceptible and *E. coli* showing the least susceptibility. This trend is consistent with previous studies that highlight the higher permeability of Gram-positive bacterial membranes to plant-derived compounds [11, 12, 13].

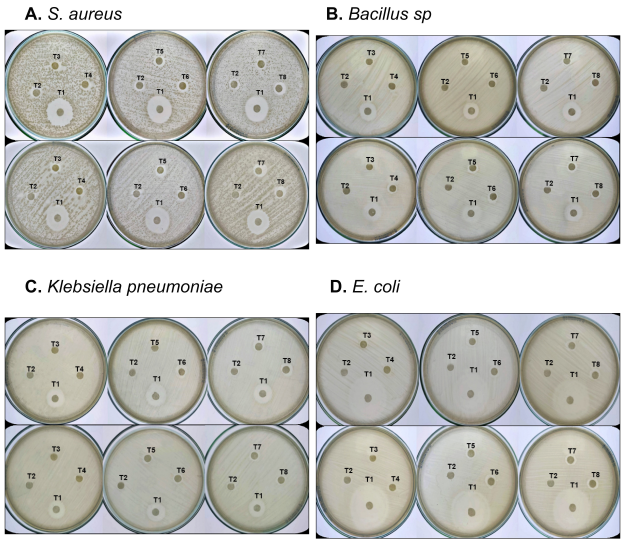

Figure 2: Antibacterial activity of ethanolic extracts of *C. urens* L. against *Staphylococcus aureus, Bacillus sp., Klebsiella pneumoniae*, and *Escherichia coli*. The figure presents inhibition zones of different dilutions of the stem extract (top) and leaf extract (bottom) in ethanol. T1: Positive control (Kanamycin for *S. aureus*, *Bacillus sp.*, and *K. pneumoniae*, Ciprofloxacin for *E. coli*), T2: Negative control (Distilled water), T3: Concentrated extract, T4: 1:1 dilution, T5: 1:2 dilution, T6: 1:3 dilution, T7: 1:4 dilution, T8: 1:5 dilution.

Statistical analysis revealed significant differences in bacterial growth inhibition among treatments, confirming the concentration-dependent nature of the extracts' antibacterial activity [14, 15]. The general linear model (GLM) showed that treatment concentration had a highly significant effect on the inhibition zones ($F = 155.73$, $p < 0.0001$). In contrast, the type of extract (leaf vs. stem) did not show a significant main effect ($p = 0.9484$), nor did the bacterial strain alone ($p = 0.0610$). However, significant interactions were found between extract type and bacterial strain ($p < 0.0001$), as well as between treatment and bacterial strain ($p = 0.0022$), suggesting that the antimicrobial response varied depending on both the extract–bacteria combination and treatment dilution.

According to the Tukey HSD test ($\alpha = 0.05$), the positive control (treatment 1) produced significantly larger inhibition zones (mean = 19.78 mm; group A) compared to all other treatments (Figure 3). Treatments 7 and 8 exhibited moderate antibacterial activity (mean diameters of 9.22 mm and 9.06 mm, respectively; group B), while treatments 3 to 6 showed significantly lower effectiveness (grouped under C and D). The negative control (treatment 2) consistently yielded the smallest inhibition zones (mean = 1.00 mm; group E), confirming its expected lack of antibacterial effect.

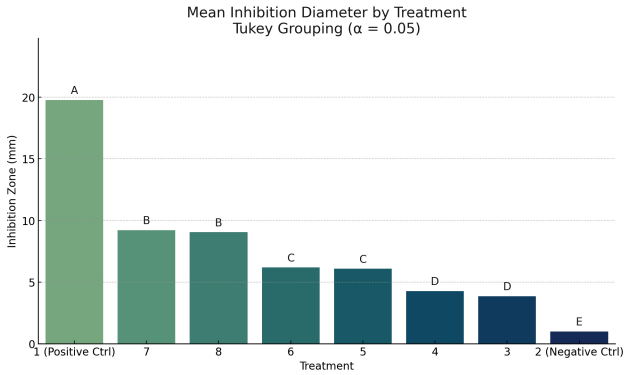

Figure 3: Mean inhibition zone diameters (mm) for each treatment, based on the overall antibacterial activity of Cnidoscolus urens extracts. Bars sharing the same letter are not significantly different (Tukey's HSD test, $\alpha = 0.05$). Treatment 1 corresponds to the positive control; treatment 2 to the negative control; treatments 3–8 represent extract dilutions from 1:0 to 1:5.

3.3 Bioactive Compounds in *C. urens* L. Extracts

Gas chromatography-mass spectrometry (GC-MS) analysis revealed differences in the chemical composition of ethanolic extracts from *C. urens* L. leaves and stems. Several biologically active compounds were identified (Table 1).

The antibacterial activity observed in this study, although modest, may be attributed to the presence of bioactive compounds such as n-hexadecanoic acid, phytol, and diethyl phthalate, which have been reported to disrupt bacterial cell membranes or affect other vital cellular processes. These lipophilic compounds can integrate into the lipid bilayer, altering membrane fluidity and permeability, which may lead to leakage of cytoplasmic contents and eventual cell death [16].

n-Hexadecanoic acid, also known as palmitic acid, has demonstrated antimicrobial activity against both bacteria and fungi. In a study by Idris *et al.* [17] this compound exhibited bacteriostatic effects against *Xanthomonas campestris*. The proposed mechanism involves disruption of the bacterial cell membrane structure due to interactions between the hydroxyl groups of lipopolysaccharides and the acid, leading to membrane asymmetry and compromised integrity. This disruption is associated with cellular lysis and loss of viability.

Phytol, a diterpene alcohol derived from chlorophyll, has

also shown potent antibacterial activity, particularly against *Pseudomonas aeruginosa*. Phytol exerts its antimicrobial effect primarily through the induction of oxidative stress. In treated cells, phytol caused a significant accumulation of intracellular reactive oxygen species (ROS), depletion of NADH, and reduction of glutathione (GSH) levels, leading to redox imbalance [18]. Phytol treatment resulted in membrane depolarization and cell filamentation, which are hallmarks of bacterial cell death. Phytol triggers multiple cytotoxic pathways, including oxidative damage to macromolecules and disruption of membrane potential.

Finally, diethyl phthalate (DEP), another compound identified in the GC–MS profile of *C. urens*, has been previously reported for its antimicrobial and antioxidant properties. Chirumamilla *et al.* (2022) identified DEP in methanolic extracts of *Solanum khasianum*, where it showed activity against both Gram-positive and Gram-negative bacteria. The proposed antimicrobial mechanism of DEP involves its ability to penetrate and disrupt microbial cell membranes, possibly by altering membrane fluidity or interfering with membrane-associated enzymes [19].

These findings align with preliminary research indicating that plant extracts exhibit broad-spectrum antimicrobial activity, which varies depending on the type of bacteria and treatment applied [20, 21]. Consequently, plant extracts may offer a viable alternative for microbial infection treatments, particularly in cases where conventional antibiotics are ineffective or contraindicated.

Further investigations are warranted to assess the safety and effectiveness of plant extracts across different populations and varied clinical settings. This study's findings suggest that leaf and stem extracts from *C. urens* possess antibacterial properties, potentially serving as therapeutic agents against bacterial infections. In the future, plant extracts may complement or even replace conventional antibiotics [22]

4 Conclusion

Our experimental results suggest that *C. urens* leaf and stem extracts have antibacterial properties, indicating potential therapeutic applications in combating bacterial infections. Despite the promising findings, more research is required to establish their effectiveness and safety across diverse populations and in various clinical settings. The potential for plant extracts to serve as adjunct or alternative solutions to conventional antibiotics makes them a promising field of research. Emphasis should be placed on further exploration of the therapeutic potential of plant extracts and development of strategies to optimize their efficacy while minimizing potential side effects. By adopting an interdisciplinary and collaborative approach, progress can be made towards the development of new antimicrobial treatments and combating antibiotic resistance.

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