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Antioxidant and antibacterial activity of *Buddleja scordioides* (Scrophulariaceae) and *Flourensia cernua* (Asteraceae) extracts

Actividad antioxidante y antibacteriana de los extractos de *Buddleja scordioides* (Scrophulariaceae) y *Flourensia cernua* (Asteraceae)

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Abstract:

Background and Aims: *Buddleja scordioides* and *Flourensia cernua* are plants traditionally used to treat gastrointestinal infection. They belong to the great diversity of medicinal plants in Mexico. The objective of this research was to determine the minimum inhibitory concentration (MIC) and phytochemical compound profile of hydroalcoholic extracts of *Buddleja scordioides* and *Flourensia cernua*.

Methods: MIC was evaluated against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Candida albicans* ATCC 10231. Total polyphenol content (TPC) was quantified using the Folin-Ciocalteu method, total flavonoid content (TFC) using the aluminum chloride colorimetric technique, and antioxidant activity (AA) using DPPH, ABTS, and FRAP. Phytochemical compounds were analyzed using HPLC-MS.

Key results: *Buddleja scordioides* showed moderate inhibition against *Candida albicans* at 1.87 mg/ml and 7.5 mg/ml, in *Staphylococcus aureus* and *Escherichia coli* it showed low inhibition at 15 mg/ml. Meanwhile, *Flourensia cernua* showed moderate activity against *Candida albicans* (3.75 mg/ml) and low inhibition against *Staphylococcus aureus* and *Escherichia coli* (15 mg/ml). The highest concentration of TPC with 76.37±1.56 mg AGE/g, and in TFC with 42.03±0.98 mg QE/g. The AA of *Flourensia cernua* in DPPH and FRAP was 132.08±2.23 mg TE/g and 128.01±1.52 mg TE/g, respectively, with higher values than in *Buddleja scordioides*. Finally, *Buddleja scordioides* showed a higher value in ABTS with 142.25±19.06 mg TE/g compared to *Flourensia cernua*. In the identification of phytochemicals, the presence of caffeic acid, quercetin, and hydroxybenzoic acid was detected as components of *Buddleja scordioides*, and carnosol and gallic acid in the extract of *Flourensia cernua*.

Conclusions: Hydroalcoholic extracts of *Buddleja scordioides* and *Flourensia cernua* showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Additionally, a high concentration of polyphenols and flavonoids led to significant antioxidant potential.

Key words: DPPH, flavonoids, hojasén, polyphenols, salvilla.

Resumen:

Antecedentes y Objetivos: *Buddleja scordioides* y *Flourensia cernua* son plantas utilizadas tradicionalmente para tratar infecciones gastrointestinales. Pertenecen a la gran diversidad de plantas medicinales en México. El objetivo de esta investigación fue determinar la concentración mínima inhibitoria (CMI) y el perfil de compuestos fitoquímicos de extractos hidroalcohólicos de *Buddleja scordioides* y *Flourensia cernua*. La CMI se evaluó contra *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 y *Candida albicans* ATCC 10231.

Métodos: El contenido total de polifenoles (CTP) se cuantificó utilizando el método de Folin-Ciocalteu, el contenido total de flavonoides (CTF) mediante la técnica colorimétrica del cloruro de aluminio, y la actividad antioxidante (AA) mediante DPPH, ABTS y FRAP. Los compuestos fitoquímicos se analizaron mediante HPLC-MS.

Resultados clave: *Buddleja scordioides* mostró inhibición moderada contra *Candida albicans* a 1.87 mg/ml y 7.5 mg/ml, mientras que, en *Staphylococcus aureus* y *Escherichia coli* presentó una baja inhibición en 15 mg/ml. Por su parte, *Flourensia cernua* mostró actividad moderada contra *Candida albicans* (3.75 mg/ml) y baja inhibición contra *Staphylococcus aureus* y *Escherichia coli* (15 mg/ml). La mayor concentración de CTP fue de 76.37±1.56 mg EAG/g y en CTF de 42.03±0.98 mg EQ/g. La AA de *Flourensia cernua* en DPPH y FRAP fue de 132.08±2.23 mg ET/g y 128.01±1.52 mg ET/g, respectivamente, con valores superiores a los de *Buddleja scordioides*. Finalmente, *Buddleja scordioides* mostró un mayor valor en ABTS con 142.25±19.06 mg ET/g en comparación con *Flourensia cernua*. En la identificación de fitoquímicos, se detectó la presencia de ácido cafeico, quercetina y ácido hidroxibenzoico como componentes de *Buddleja scordioides*, y carnosol y ácido gálico en el extracto de *Flourensia cernua*.

Conclusiones: Los extractos hidroalcohólicos de *Buddleja scordioides* y *Flourensia cernua* mostraron actividad antimicrobiana contra *Escherichia coli*, *Staphylococcus aureus* y *Candida albicans*. Además, una alta concentración de polifenoles y flavonoides condujo a un potencial antioxidante significativo.

Palabras clave: DPPH, flavonoides, hojasén, polifenoles, salvilla.

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Introduction

Currently, public health is facing an alarming situation due to the increasing occurrence of antimicrobial resistance (AMR). According to the Pan American Health Organization (PAHO, 2021), it is estimated that about 7,000,000 deaths occur each year due to AMR, and for the next 25 years, this figure can reach up to 10 million.

AMR is a biological phenomenon that occurs naturally in microorganisms as an adaptive response to the surrounding environment. Resistance mechanisms can occur naturally or be acquired. The first arises in the absence of factors that force microorganisms to adapt or evolve to survive and is due to their natural structure or functional features. Acquired resistance is characterized by the generation of changes, mutations, or gene gains in the microorganism's composition in response to adaptation. One of the resistance mechanisms occurring in microorganisms is decreased bacterial permeability (Belay et al., 2024; Ho et al., 2025). This reduced uptake is generated by limiting drug entry through alterations to the structure, either by modifying or inactivating the antibiotic binding sites (Camacho Silvas, 2023; SADER, 2023). As a result, microbial agents stop responding to antimicrobial medicines used to treat infections (WHO, 2023). Currently, antibiotics, antivirals, and antifungal drugs used in the treatment of infectious diseases can be applied in excess or, in some cases, unnecessarily, leading to antimicrobial resistance (WHO, 2021). In 2019, 1.3 million deaths were reported from infections caused by resistant microorganisms, a figure higher than the number of deaths caused by AIDS and malaria (Naghavi, 2022; UNEP, 2023). Microbial resistance increases the likelihood of the microbe spreading, complicates treatment, and raises mortality and medical expenses associated with treatment (Rocha et al., 2018; COFEPRIS, 2021). Against this backdrop, it is essential to develop new antimicrobial drugs that treat and slow down the spread of resistant microorganisms.

In recent studies, various parts of plants, including leaves, roots, bark, fruits, and seeds, have been report-

ed to contain phytochemicals with beneficial activities for treating different diseases (Haruna and Yahaya, 2021). Approximately 67% of medicines originate from nature, with 25% derived from plants (Gallegos-Zurita, 2016). Plant products are also commonly used to treat inflammation, hypertension, digestive problems, respiratory diseases, kidney and liver disorders, diabetes mellitus, and wound healing (dos Santos et al., 2023). Likewise, many studies employ plants as antimicrobial treatments (Hemeg et al., 2020; Leos-Rivas et al., 2024; Osorno-Sánchez et al., 2025).

Buddleja scordioides Kunth, commonly known as escobilla butterflybush (Spanish: salvilla), and *Flourensia cernua* DC., commonly known as American tarwort (Spanish: hojasén), are representative of the great diversity of medicinal plants of the Mexican desert area. They are commonly used in traditional medicine to treat different health conditions. *Buddleja scordioides* is active against gastrointestinal problems that present symptoms related to pain, inflammation, and diarrhea (Villegas-Novoa et al., 2020). *Flourensia cernua* has been used as a laxative, expectorant, and antirheumatic. Its antioxidant and antifungal capacities have also been established (Álvarez-Pérez et al., 2020).

These two plants are known for their beneficial health properties. However, studies on antimicrobial activity are still limited, indicating an opportunity to explore their potential as a source of new antimicrobial agents. Therefore, this research proposes to determine the antimicrobial activity of hydroalcoholic extracts of *Buddleja scordioides* and *Flourensia cernua* against common pathogenic bacteria (*Escherichia coli* (Migula, 1895) Castellani & Chalmers, 1919 (*E. coli*, ATCC 25922) and *Staphylococcus aureus* subsp. *aureus* (Rosenbach, 1945) (*S. aureus*, ATCC 25923) and a fungus (*Candida albicans* (C.P. Robin) Berkhout (*C. albicans*, ATCC 10231). In addition, polyphenol content is quantified, the components are identified, and the antioxidant capacity of the extracts is established.

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Materials and Methods

Chemicals

Folin Ciocalteu reagent (sodium 3,4-dioxi-3,4-dihydronaphthalene-1-sulfonate), Trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-pyridylhydrazil), gallic acid (3,4,5-trihydroxybenzoic acid), ABTS (2,2'-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid), TPTZ (2,4,6-tripyridyls-triazine), acetate buffer and quercetin were purchased from Sigma-Aldrich Co. (St. Louis, USA). Ethanol, methanol, sodium hydroxide, aluminum chloride and potassium acetate were obtained from Merck, Mexico. Ferric chlorides ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) came from Jalmerk Cientifica S.A. de C.V (San Nicolás de los Garza, Mexico).

Collection of plant material

In May 2022, the species *Buddleja scordioides* and *Flourensia cernua* were collected near farmland in the rural area of Cuencamé, Durango, Mexico, at the following coordinates: 24°52'14"N, 103°41'48"W. Thirty-eight percent of the soil in this area is Phaeozem, which is characterized by being dark, rich in organic matter, and having high agricultural potential (SEMARNAT, 2008; INEGI, 2010).

The plants were collected during the flowering season and corresponded to mature individuals in the reproductive stage. Once the material was collected, it was identified in the Department of Agroecology, Universidad Autónoma Antonio Narro, Unidad Laguna, through visual inspection and comparison with the reference herbarium database of the Regional Botanical Reference Center. A reference specimen of each collection was also preserved in the herbarium CRB-Agroecología (03/022). The identified plant species and the anatomical structure is shown in Fig. 1. The stems and leaves of the plants were used to prepare the extracts.

Extract preparation

The plant material was air-dried and then pulverized using mechanical force with a mill (Nixtamatic, NG-02, CDMX, Mexico) and a blender (Oster, BLSTBPST013, Cd. Acuña, Mexico) depending on the hardness of the plant part. For the hydroalcoholic extraction, 5 g of the pulverized plant material was weighed, added to 30 ml of ethanol 70%, and

the mixture was macerated for 72 h in an amber jar at room temperature (RT). Subsequently, the extract was centrifuged (ThermoFisher, Waltham, MA, USA) for 5 minutes at RT and 10,000 rpm. The supernatant obtained was placed in a stove (BLUE M SW-11TA, Blue Island, IL, USA) at 45 to 50°C until dry. Finally, the dry extract was stored in an amber jar at 4°C for later use (Surveswaran et al., 2007; Ramírez-Moreno et al., 2016).

Antimicrobial activity

Certified strains

The certified strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Candida albicans* ATCC 10231 were preserved with saline and glycerol solution at -20°C. The strains were subcultured in tubes with Brain Heart Infusion (BHI) agar.

Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the serial dilution method. The microbial suspensions of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were adjusted to 0.5 on the McFarland scale. Each extract was evaluated individually, and a stock solution of each extract was prepared at 60 mg/ml, from which six serial dilutions were made covering a range of 30 mg/ml to 0.9375 mg/ml. Four control tubes with BHI broth were included. In two of them, 100 µl of the reference antimicrobial agents was added: ciprofloxacin (500 mg) for bacterial strains and fluconazole (300 mg) for fungal strains were added, both at a final concentration of 1.2 mg/ml and used as growth inhibition controls in the third tube, 100 µl of the microbial strain and 100 µl of ethanol were added and used as a viability growth control. In the fourth tube, only 100 µl of ethanol was added, and it was used as a blank control. The tubes were then incubated (Quincy Lab, 12-140, IL, USA) at 37 °C for 24 hours. Finally, a loopful from the previously incubated tubes was collected using a sterile calibrated bacteriological loop and streaked onto Mueller-Hinton agar plates for bacteria and nutrient agar for yeasts. The plates were incubated at 37 °C for 24 hours. The results were expressed as MIC, which is defined



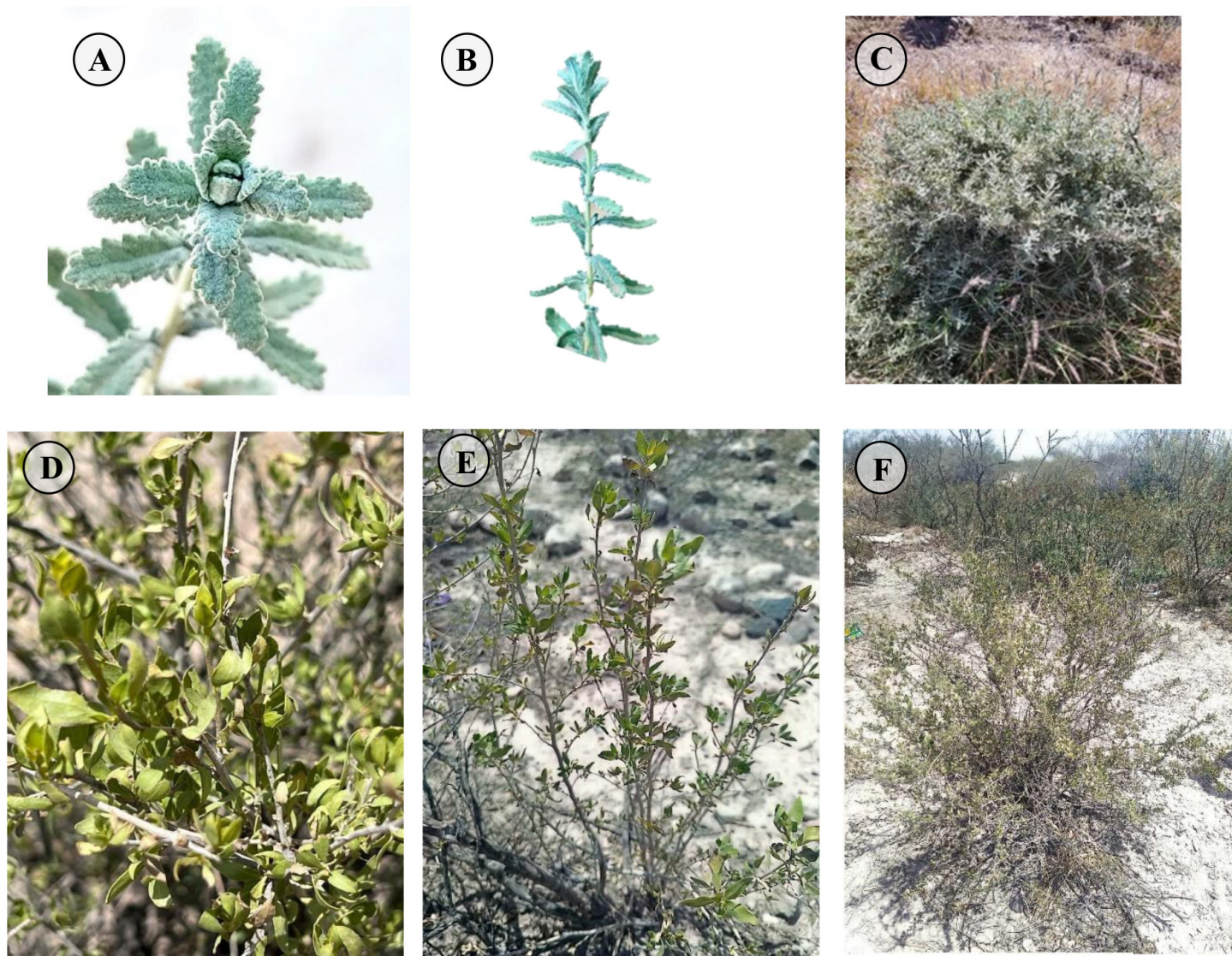


Figure 1: A-C. *Buddleja scordioides* Kunth; A. leaves; B. stem; C. shrub. D-F. *Flourensia cernua* DC.; D. leaves; E. stems; F. shrub. Photos: Adriana Carolina González Machado.

as the minimum concentration required to inhibit microbial growth (Tolosa and Cañizares, 2002; Mogana et al., 2020). As a complementary analysis, the susceptibility profiles of the tested strains against ciprofloxacin and fluconazole were determined using the automated VITEK 2XL system (bioMérieux, Marcy-l'Étoile, France). This system provides minimum inhibitory concentration (MIC) values along with their corresponding clinical categorization (susceptible, intermediate, or resistant), according to standardized antimicrobial susceptibility criteria. The values obtained were used as comparative references to contextualize the antimicrobial activity observed in the plant extracts, allowing

for a contrast with clinically used antimicrobial agents and strengthening the interpretation of results.

Phytocompound determination

One mg of the extract was dissolved in 1 ml of purified water (drinking-grade) and was used for phytocompound analysis.

Quantification of total polyphenols (TPC)

One hundred and fifty μ l of the extract sample at 1000 ppm was mixed with 150 μ l of Folin's 0.2 N reagent. Then, 300 μ l of 0.35 N NaOH solution was added, and the mixture was



incubated for 5 min at RT in the dark. Absorbance was measured in a spectrophotometer (Velab, Pharr, TX, USA) at 760 nm (Magalhães et al., 2010; Palma-Wong et al., 2023). A standard gallic acid curve was prepared with gallic acid concentrations ranging from 7.81 to 250 mg/ml. The values were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

Flavonoids evaluation (CFT)

One ml of the extract at 1000 ppm was mixed with 3 ml of ethanol, then 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water were added. The mixture was incubated at RT in the dark for 30 minutes. The samples were read at 420 nm (Velab, Pharr, TX, USA). A standard curve of quercetin was prepared with quercetin concentrations ranging from 7.81 to 250 mg/ml. The results were expressed as milligrams quercetin equivalent of quercetin per gram of extract (mg QE/g) (Iraqi et al., 2013; González-Machado et al., 2023).

Antioxidant activity evaluation (AA)

DPPH method

Here, 0.1 ml of extract at 1000 ppm was mixed with 3.9 ml of DPPH at 6×10^{-5} M. The mixture was incubated for 30 minutes in the dark at RT. Absorbance was measured in a spectrophotometer (Velab, Pharr, TX, USA) at 518 nm. A standard curve of Trolox was prepared with Trolox concentrations ranging from 7.81 to 500 mg/ml. The values were expressed as milligrams of Trolox equivalent per gram of extract (mg TE/g) (Brand-Williams et al., 1995; González-Díaz and García-Nuñez, 2023).

FRAP method

The FRAP reagent was prepared by mixing 1:1:10 ratios of the following: FeCl_3 (20 mM dissolved in water), TPTZ (40 mM dissolved in 40 mM HCl), and 0.3 M acetate buffer (pH 3.6). FRAP solution was prepared on the test day and incubated at 37 °C for 10 min before use. For the assay, 75 μl of 0.1 ml of extract was mixed of extract at 1000 ppm was mixed with 1.425 μl of FRAP solution. The mixture was incubated in the dark for 10 min at 37 °C, and absorbance was measured at a wavelength of 593 nm (Velab,

Pharr, TX, USA). Before the trial, a standard Trolox curve (7.81 to 500 $\mu\text{g}/\text{ml}$) was prepared. The values were expressed as milligrams of Trolox equivalent per gram of extract (mg TE/g) (Benzie and Strain, 1996; Benítez-Estrada et al., 2020; Palma-Wong et al., 2023).

ABTS method

The ABTS+ reagent solution was generated by mixing 7 mM ABTS with 2.25 mM potassium persulfate (1:1) and incubating for 12 to 18 h at RT. The ABTS+ solution was diluted with ethanol (1:40) and adjusted to an absorbance of 0.7 ± 0.02 at 734 nm in a spectrophotometer (Velab, Pharr, TX, USA). For sample measurement, 10 μl of the extract at 1000 ppm was mixed with 1000 μl of ABTS+ reagent, allowed to react for 1 min, and then read at 734 nm. A standard Trolox curve was previously prepared using concentrations of 7.81 to 500 $\mu\text{g}/\text{ml}$. The results were expressed as milligrams of Trolox equivalent per gram of extract (mg TE/g) (Re et al., 1999; Torres-León et al., 2021).

Identification of phytochemicals by HPLC-MS

Phytochemical identification was performed using reversed-phase high-performance liquid chromatography (HPLC) with a Varian HPLC system, which included an autosampler (Varian ProStar 410, Walnut Creek, CA, USA) and a ternary pump (Varian ProStar 2301, Walnut Creek, CA, USA). An ion-trap mass spectrometer (Varian 500-MS IT Mass Spectrometer, Walnut Creek, CA, USA) was also used, equipped with an electrospray ion source. The samples (5 μl) were injected into a Denali C18 column (150 mm $\times$ 2.1 mm, 3 μm , Grace, USA), keeping the oven temperature at 30 °C. The eluents used were formic acid (0.2%, v/v; solvent A) and acetonitrile (solvent B), with the following gradient: first 3% B; from 0 to 5 minutes, linear increase up to 9% B; from 5 to 15 minutes, linear increase up to 16% B; and from 15 to 45 minutes, linear increase up to 50% B. After each analysis, the column was washed and reconditioned. The flow rate remained constant at 0.2 ml/min, and detection was performed at wavelengths of 245, 280, 320, and 550 nm. The total eluent (0.2 ml/min) was directed to the source of the mass spectrometer, without fractionation. All mass spectrometry experiments were performed in negative mode (M⁻/H), using nitrogen



as the nebulizer gas and helium as the entrainment gas. First, analysis was performed in full scan mode, covering the statistical range of m/z 50-2000 (Ascacio-Valdés et al., 2016).

Statistical analysis

All analyses were conducted in triplicate. The values were expressed as the mean with their standard deviation. An ANOVA was performed with a 95% confidence level, considering values significant at $p < 0.05$. The data were subject to mean comparisons using Tukey's test. Calculations and analyses were performed using IBM SPSS v. 23 software (IBM SPSS, 2023).

Results

The antimicrobial activity results are shown in table 1. In general terms, both extracts showed inhibitory activity against the three microbial species analyzed. The *Buddleja scordioides* extract had the greatest antimicrobial effect against *Candida albicans*, with a MIC of 1.87 mg/ml, while *Flourensia cernua* showed lower values, although it also inhibited its growth. With regard to *Escherichia coli*, both *Buddleja scordioides* and *Flourensia cernua* were able to inhibit bacterial growth at a concentration of 15 mg/ml. In the case of *Staphylococcus aureus*, *Buddleja scordioides* showed greater efficacy, with a MIC of 7.5 mg/ml, in contrast to *Flourensia cernua*, which reached an MIC of 15 mg/ml.

With regard to secondary metabolite content, table 2 shows the results for total phenolic content (TPC)

and total flavonoid content (TFC). The extract of *Buddleja scordioides* recorded a TPC of 76.37 ± 1.56 mg GAE/g and a TFC of 42.03 ± 0.98 mg QE/g, positioning it as the species with the highest concentration of these compounds. *Flourensia cernua*, on the other hand, had a similar TPC (73.23 ± 1.10 mg GAE/g), but a considerably lower TFC with 15.93 ± 0.71 mg QE/g).

In addition, table 2 presents the antioxidant activity (AA) results obtained using the DPPH, FRAP, and ABTS. The extract of *Flourensia cernua* showed the highest antioxidant potential in the DPPH (132.08 ± 2.23 mg TE/g) and FRAP (128.01 ± 1.52 mg TE/g) methods, while *Buddleja scordioides* obtained the highest value in ABTS (142.25 ± 19.06 mg TE/g). These data suggest that, although both species have high antioxidant activity, the type of assay determines which extract is most effective.

Finally, phytochemical analysis by HPLC-MS revealed the presence of various bioactive compounds in both extracts (Figs. 2, 3). In *Buddleja scordioides*, 10 phytochemicals belonging to different chemical families were identified, notably caffeic acid, quercetin, and hydroxybenzoic acid, all of which have been previously reported to have antimicrobial activity (Kępa et al., 2018; Guburzer, 2021; Wang et al., 2023). In *Flourensia cernua*, 17 compounds were detected, including caffeic acid, carnosol, and gallic acid, which have been associated with antibacterial and fungicidal effects in previous studies (Vázquez et al., 2016; Kępa et al., 2018; Liberato et al., 2022). Table 3 and table 4 show the phytochemical composition of each extract.

Table 1: Minimum inhibitory concentration (MIC) of hydroalcoholic extracts of *Buddleja scordioides* Kunth and *Flourensia cernua* DC. against *Escherichia coli* (Migula, 1895) Castellani & Chalmers, 1919 (*E. coli*, ATCC 25922) and *Staphylococcus aureus* subsp. *aureus* (Rosenbach, 1945) (*S. aureus*, ATCC 25923) and a fungus (*Candida albicans* (C.P. Robin) Berkhout (*C. albicans*, ATCC 10231) expressed in mg/ml. N/A: Not applicable.

Antimicrobial	<i>Candida albicans</i> (C.P. Robin) Berkhout	<i>Staphylococcus aureus</i> (Rosenbach, 1945)	<i>Escherichia coli</i> (Migula, 1895) Castellani & Chalmers, 1919
Extracts			
<i>Buddleja scordioides</i> Kunth	1.87	7.5	15
<i>Flourensia cernua</i> DC.	3.75	15	15
Standard antimicrobial controls			
Ciprofloxacin	N/A	0.5	0.06
Fluconazole	0.5	N/A	N/A



Table 2: Total polyphenol content expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g), flavonoid expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g) and Antioxidant Activity by DPPH, FRAP, and ABTS expressed in milligrams of Trolox equivalent per gram of extract (mg TE/g) of the hydroalcoholic extracts of *Buddleja scordioides* Kunth and *Flourensia cernua* DC. Different letters indicate a significant difference ($p < 0.05$).

Extract	Phytochemical content		Antioxidant activity (mg TE/g)		
	Polyphenols (mg GAE/g)	Flavonoids (mg QE/g)	DPPH	FRAP	ABTS
<i>Buddleja scordioides</i> Kunth	76.37±1.56 ^a	42.03±0.98 ^a	125.22±8.24 ^a	98.43±4.68 ^b	142.25±19.06 ^a
<i>Flourensia cernua</i> DC.	73.23±1.10 ^a	15.93±0.71 ^b	132.08±2.23 ^a	128.01±1.52 ^a	125.38±0.31 ^a

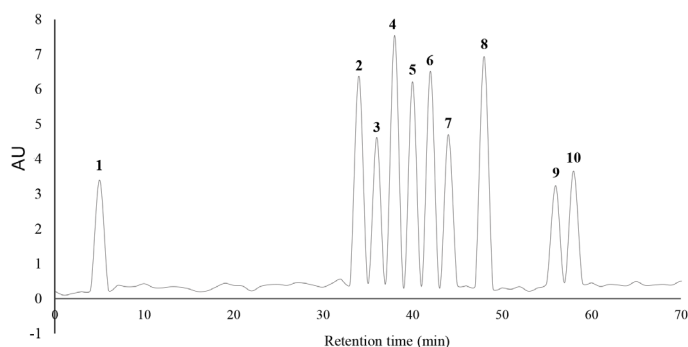


Figure 2: Phytocompounds present in *Buddleja scordioides* Kunth identified by HPLC-MS. Numbers assigned to each peak correspond to the compounds described in table 3. AU=Absorbance units.

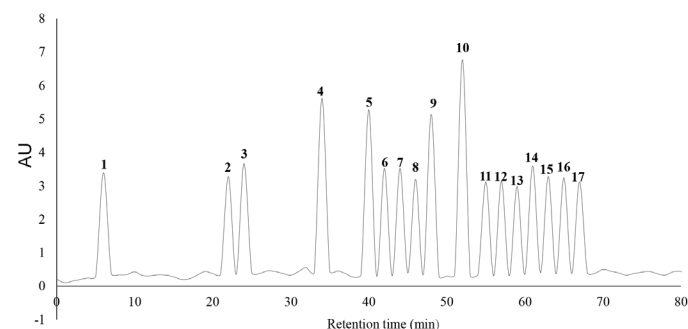


Figure 3: Phytocompounds present in *Flourensia cernua* DC. identified by HPLC-MS. Numbers assigned to each peak correspond to the compounds described in table 4. AU=Units of absorbance.

Discussion

The extracts of *Buddleja scordioides* and *Flourensia cernua* showed antimicrobial activity against Gram-positive and Gram-negative bacteria and yeasts, which is consistent

with previous reports on the efficacy of ethanolic extract of *Flourensia cernua* against fungi and bacteria. Bejarano-Castro et al. (2019) and Jasso de Rodriguez et al. (2012; 2017) demonstrated that ethanolic extract of this species possesses antifungal and antibacterial properties. However, to date, no studies on the antimicrobial activity of *Buddleja scordioides* have been documented, highlighting the originality of these findings.

It has been mentioned that the lower the MIC, the better the antimicrobial activity. The antimicrobial activity of plant extracts has been classified as good when the MIC is less than 0.1 mg/ml, moderate from 0.1 to 0.625 mg/ml, and weak at values above 0.625 mg/ml (Famuyide et al., 2019; Zouine et al., 2024).

In this study, *Buddleja scordioides* showed antimicrobial activity against *Candida albicans* (1.87 mg/ml) and *Staphylococcus aureus* (7.5 mg/ml), while inhibition of *Escherichia coli* was observed at 15 mg/ml corresponding to low activity according to the established classification criteria. *Flourensia cernua* also exhibited low antimicrobial activity against *Candida albicans* (3.75 mg/ml), *Staphylococcus aureus* (15 mg/ml), and *Escherichia coli* (15 mg/ml).

In previous studies, Jasso de Rodríguez et al. (2017) reported that the ethanolic extract of *Flourensia cernua* inhibited *Fusarium oxysporum* Schltdl., obtaining MICs of 2163 mg/l in leaves and 4240 mg/l in branches. In contrast, the present study determined much lower MIC, which is explained by the fact that it is a different fungus. Similarly, Molina-Salinas et al. (2006) reported that hexane extracts of *Flourensia cernua* showed MICs of 50 and 25 µg/ml against sensitive and resistant strains of *Mycobacterium tuberculosis* Zopf, 1883, demonstrating the versatility of this



Table 3: Chemical name and family of compounds identified from the hydroalcoholic extract of *Buddleja scordioides* Kunth. [M-H]⁻: Molecular mass.

Number	Compound	Family	[M-H] ⁻	Retention time (min)
1	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids	340.9	5.93
2	Luteolin 7-O-diglucuronide	Flavonoids	638.7	34.55
3	Myricetin 3-O-rhamnoside	Flavonols	462.8	36.83
4	Kaempferol 3-O-glucosyl-rhamnosyl-galactoside	Flavonols	754.7	37.11
5	Kaempferol 3-O-glucosyl-rhamnosyl-glucoside	Flavonols	622.8	39
6	Quercetin 3-O-(6''-acetyl-galactoside) 7-O-rhamnoside	Flavonols	652.7	42.76
7	4-hydroxybenzoic acid 4-O-glucoside	Hydroxybenzoic acids	474.7	44.31
8	1,2-Diferuloylgentiobiose	Methoxycinnamic acids	694.7	45.71
9	Feruloyl tartaric acid	Methoxycinnamic acids	324.9	56.41
10	3-feruloylquinic acid	Methoxycinnamic acids	366.8	57.98

Table 4: Chemical name and family of compounds identified from the hydroalcoholic extract of *Flourensia cernua* DC. [M-H]⁻: Molecular mass.

Number	Compound	Family	[M-H] ⁻	Retention time (min)
1	Caffeic acid 4-O-glucoside	Hydroxycinnamic acids	340.8	6.2
2	Carnosol	Phenolic terpenes	328.8	22.98
3	3-feruloylquinic acid	Methoxycinnamic acids	367.9	24.39
4	Apigenin arabinoside-glucoside	Flavonoids	562.7	34.71
5	Malvidine 3-O-galactoside	Antocyanins	528.8	40.03
6	1-caffeoylquinic acid	Hydroxycinnamic acids	352.9	40.76
7	3-caffeoylquinic acid	Hydroxycinnamic acids	352.8	42.48
8	Gallic acid 3-O-gallate	Hydroxybenzoic acids	320.9	43.41
9	1,3-Dicaffeoylquinic acid	Hydroxycinnamic acids	514.7	43.78
10	Chrysoeriol 7-O-(6''-malonyl-apiosyl-glucoside)	Methoxyflavone	678.6	46.92
11	Cirsimaritin	Dimethoxyflavone	312.8	49.35
12	Rhamnetina	O-methylated flavonol	314.8	53.21
13	Hispidulin	Methoxyflavone	298.7	55.92
14	Lariciresinol	Lignans	360.7	56.86
15	Rosmarinic Acid	Hydroxycinnamic Acids	328.8	58.36
16	Feruloyl Tartaric Acid	Methoxycinnamic Acids	324.9	58.64
17	Cirsimaritin	Dimethoxyflavone	312.8	59.34

species against different pathogens. In contrast, the anti-biogram results demonstrated that the isolates of *Staphylococcus aureus* and *Escherichia coli* were susceptible to ciprofloxacin, with MIC values <0.5 mg/ml and <0.06 mg/ml, respectively. Likewise, *Candida albicans* exhibited susceptibility to fluconazole, with growth inhibition observed at concentrations <0.5 mg/ml, indicating a high sensitivity of the yeast to this antifungal agent. These values are considerably lower than those obtained for the plant ex-

tracts, highlighting the greater potency of the conventional antibiotic. However, although conventional antimicrobials such as ciprofloxacin are highly effective in controlling bacterial infections, their use has been associated with adverse effects on health, such as gastrointestinal disorders, alteration of the composition of the intestinal microbiota (dysbiosis), and the elimination of beneficial microorganisms along with the target pathogens (Cusumano et al., 2025; Zimmerman and Cutis, 2019). In addition, continued



exposure to antibiotics has contributed to the emergence and spread of antimicrobial resistance in various clinical pathogens, posing a serious global public health problem (Contreras-Omaña et al., 2021).

Therefore, despite the relatively weak activity observed in the extracts under test conditions, the inhibitory effects detected suggest the presence of bioactive compounds that could contribute to antimicrobial action, with a potentially lower profile of adverse effects than synthetic antibiotics. Plant-derived compounds offer great potential as a source of new antimicrobial agents or as adjuvants to improve the efficacy of existing treatments, due to their multiple mechanisms of action and history of traditional use in medicine (Angelini, 2024). However, it is necessary to strengthen research focused on the identification, characterization, and mechanism of action of antimicrobial compounds in medicinal plants in order to rigorously evaluate them and guide their safe and effective application in future clinical and therapeutic studies (Woo et al., 2023).

With regard to phenolic content, TPC includes polyphenols such as phenolic acids, lignans, and stilbenes, varying according to their structure and -OH groups present (Marín-Canul et al., 2023). However, the TPC and TFC values obtained in this study are consistent with those reported by Jasso de Rodríguez et al. (2017), who reported similar contents for ethanolic extracts of *Flourensia cernua*. The variation in the amounts of polyphenols and flavonoids depends on factors such as the type of extraction and environmental conditions, since these compounds are secondary metabolites that are generated in response to stress (Naz et al., 2020; Slimani et al., 2025).

The role of polyphenols and flavonoids in the biological activity of plants has been widely documented. In addition to being related to antioxidant properties (Bonilla-Landa et al., 2022; Martínez-Mendoza et al., 2023), these compounds hinder the growth of pathogens, inhibit oxidation reactions, and delay microbial proliferation (Domingo and López-Brea, 2003). In addition, flavonoids have specific mechanisms of action, such as altering bacterial motility, inhibiting nucleic acid synthesis, disrupting the cytoplasmic membrane, interfering with energy metabolism, and inhibiting biofilm formation (Rodríguez Pérez et al., 2020).

The high antioxidant capacity observed in *Flourensia cernua* is consistent with previous studies (Álvarez-Pérez et al., 2020; Aranda-Ledesma et al., 2022). In the ABTS assay, *Buddleja scordioides* showed a higher value. To date, no studies have reported the antioxidant activity of *Buddleja scordioides* determined by the FRAP assay using the same units as those employed in the present study, limiting quantitative comparison of the results. However, Macías-Cortéz et al. (2022) evaluated aqueous extracts of *Buddleja scordioides* obtained by ultrasound-assisted extraction and reported FRAP antioxidant activity values ranging from 86.3 ± 5.3 to 249 ± 15.2 mM TE/mg of extract. Antioxidant activity is linked to hydrogen atom donation and the resonance capacity of aromatic rings, which allows free radicals to be neutralized and metals to be chelated (Zagoskina et al., 2023). These properties not only explain the protective effect against oxidative stress but can also enhance antimicrobial activity, as AA has been documented to contribute to the inhibition of enzymes and processes associated with microbial pathogenicity (Pham, 2008; Cárdenas-Perea et al., 2014; Vargas-Sánchez et al., 2014; García-González et al., 2019).

Finally, the compounds identified by HPLC-MS reinforce the explanation of the biological effects observed. The physiological properties of plants are attributed to the presence of specific compounds for each therapeutic property, specifically in antimicrobial and antioxidant activity, which is related to the combined effects of polyphenols (Negi, 2012). In this context, kaempferol (*Flourensia cernua*) and quercetin (*Buddleja scordioides*) were the major compounds identified in the present study.

In *Buddleja scordioides*, quercetin exhibits a high free radical scavenging capacity in DPPH and ABTS assays, which is attributed to its phenolic hydroxyl groups, a catechol structure that enhances its antioxidant efficacy (Qi et al., 2022; Baqer et al., 2024). Moreover, its antimicrobial activity against Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, is associated with disruption of membrane integrity, inhibition of nucleic acid synthesis, and attenuation of virulence factors. These mechanisms have been linked to enzyme inhibition and membrane destabilization (Villegas-Novoa et al., 2020; Elumalai et al., 2022; Nguyen and Bhattacharya, 2022; Baqer et al., 2024).



Additionally, other compounds with antimicrobial potential were identified. Caffeic acid may alter cellular permeability by affecting membrane hydrophobicity and causing potassium ion leakage (Khan et al., 2021), whereas hydroxybenzoic acid enhances antimicrobial activity due to its lipophilicity (Manuja et al., 2013).

In *Flourensia cernua*, kaempferol has been reported to compromise bacterial cytoplasmic membrane integrity, alter cellular permeability, inhibit enzymes involved in DNA replication and essential metabolic pathways, and interfere with biofilm formation (Calderón-Montaña et al., 2011; Tatsimo et al., 2012). Its antioxidant activity is mainly attributed to its ability to donate hydrogen atoms or electrons to neutralize reactive oxygen species (ROS), as well as to chelate pro-oxidant metal ions such as Fe^{2+} , thereby limiting Fenton-type reactions. Other compounds present in the extract, such as carnosol, are notable for their ability to inhibit *Staphylococcus aureus* and reduce biofilm formation (Shen et al., 2020), while gallic acid has been described as an inhibitor of bacterial antioxidant enzymes, promoting ROS accumulation and oxidative damage (Carvalho et al., 2018). Overall, these findings strengthen the interpretation of the antioxidant and antimicrobial effects observed in the evaluated extracts. Together, these findings demonstrate that the antimicrobial and antioxidant properties of *Buddleja scordioides* and *Flourensia cernua* are closely related to the presence of polyphenols and flavonoids which depend on both environmental factors and extraction conditions (Ramírez-Rojo et al., 2018; Li and Ahammed, 2023).

Conclusions

The extracts of *Buddleja scordioides* and *Flourensia cernua* exhibited low to moderate antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. A high concentration of polyphenols and flavonoids resulted in significant antioxidant activity, as measured by the radical scavenging methods of DPPH, FRAP, and ABTS. Among the various phytochemicals identified by HPLC-MS are caffeic acid, quercetin, hydroxybenzoic acid, carnosol, and gallic acid, which, according to the literature, are responsible for antioxidant activity. To better understand the antimicrobial action of the compounds extracted from *Buddleja scordioides* and *Flourensia cernua*, further *in vi-*

tro and *in vivo* assays are needed, employing optimization techniques to increase the concentration of the identified phenolic compounds, as well as detailed pharmacological analyses. While the results indicate relevant antioxidant properties, the low and moderate antimicrobial activity observed suggests that further validation studies are needed before considering potential applications in the pharmaceutical and food industries.

Author contributions

Conceptualization: ARM; Data curation: JAAV; Formal analysis: DPE; Investigation and methodology: ARM, LRS; Methodology: ICM; Writing - original draft: ACGM, JEM; Writing - review and editing: ARM, CTL.

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Data Availability Statement

The dataset that supports the results of this study was published in SciELO Data and can be accessed at DOI: <https://doi.org/10.48331/SCIELODATA.DPVOLF>

Use of Artificial Intelligence

The authors declare that artificial intelligence was not used at any stage of the research, nor in editing or stylistic revision tasks.

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