Antimicrobial activity of ethanol extracts of *Sambucus canadensis, Bidens alba, Ricinus communis* and *Salvia microphylla* against foodborne pathogens

Atividade antimicrobiana de extratos etanólicos de *Sambucus canadensis, Bidens alba, Ricinus communis* e *Salvia microphylla* contra patógenos de origem alimentar

Actividad antimicrobiana de extractos de etanol de *Sambucus canadensis, Bidens alba, Ricinus communis* y *Salvia microphylla* contra patógenos transmitidos por alimentos

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ABSTRACT
Bacteria that compromise food safety cause serious diseases globally, with a higher recurrence in developing countries. This has underscored the need to explore effective antimicrobial alternatives. This study aimed to evaluate the antimicrobial activity of ethanolic extracts from *Sambucus canadensis*, *Bidens alba*, *Ricinus communis* and *Salvia microphylla* against Gram-positive and Gram-negative bacteria through both *in vitro* and *in vivo* tests. Phytochemical analyses and functional group identification using IR spectroscopy were performed. *In vitro* testing utilized disk diffusion and microplate techniques, while *in vivo* testing involved *Solanum lycopersicum* to assess microbial loads within the maximum allowable limit (MAL) according to the Official Mexican Standard NOM-093-SSA1-1994. Phytochemical and IR analyses confirmed the presence of flavonoids, terpenes, and saponins, all of which are known to possess antimicrobial properties. Overall, all extracts inhibited both Gram-positive and Gram-negative bacteria. Notably, the extracts of *S. canadensis* and *B. alba* exhibited the largest zones of inhibition, which were statistically significant ($P<0.05$) compared to the other extracts and the control. Minimum inhibitory concentration (MIC) values ranged from 68.1 to 1000 µg mL^{-1}, with the *Sambucus canadensis* extract showing the greatest inhibition against *Staphylococcus aureus* and *Klebsiella pneumoniae* at 68.1 and 75.1 µg mL^{-1}, respectively. *In vivo* tests demonstrated that all extracts reduced the bacterial load of the evaluated strains, except for *Salmonella typhimurium.* These findings suggest that the evaluated extracts could be a promising alternative for natural antimicrobials against foodborne pathogens that threaten food safety and human health.

Keywords: natural antimicrobials, medicinal plants, bacteria, bacterial resistance.

RESUMO
Bactérias que comprometem a segurança alimentar causam doenças graves globalmente, com maior recorrência em países em desenvolvimento. Isso ressaltou a necessidade de explorar alternativas antimicrobianas eficazes. Este estudo teve como objetivo avaliar a atividade antimicrobiana de extratos etanólicos de Sambucus canadensis, Bidens alba, *Ricinus communis* e *Salvia microphylla* contra bactérias Gram-positivas e Gram-negativas por meio de testes *in vitro* e *in vivo*. Análises fitoquímicas e identificação de grupos funcionais usando espectroscopia de infravermelho foram realizadas. Os testes *in vitro* utilizaram técnicas de difusão em disco e microplacas, enquanto os testes *in vivo* envolveram Solanum lycopersicum para avaliar cargas microbianas dentro do limite máximo permitido (MAL) de acordo com o Padrão Oficial Mexicano NOM-093-SSA1-1994. As análises fitoquímicas e de infravermelho confirmaram a presença de flavonoides, terpenos e saponinas, todos conhecidos por possuírem propriedades antimicrobianas. No geral, todos os extratos inibiram bactérias Gram-positivas e Gram-negativas. Notavelmente, os extratos de *S. canadensis* e *B. alba* exibiram as maiores zonas de inibição, que foram estatisticamente significativas ($P<0,05$) em comparação aos outros extratos e ao controle. Os valores de concentração inibitória mínima (CIM) variaram de 68,1 a 1000 µg mL^{-1}, com o extrato de Sambucus canadensis mostrando a maior inibição contra *Staphylococcus aureus* e *Klebsiella pneumoniae* em 68,1 e 75,1 µg mL^{-1}, respectivamente. Testes *in vivo* demonstraram que todos os extratos reduziram a carga bacteriana das cepas avaliadas, exceto *Salmonella typhimurium*. Essas descobertas sugerem que os extratos avaliados podem ser uma alternativa promissora para antimicrobianos naturais contra patógenos de origem alimentar que ameaçam a segurança alimentar e a saúde humana.

Palavras-chave: antimicrobianos naturais, plantas medicinais, bacterias, resistência bacteriana.

RESUMEN
Las bacterias que comprometen la seguridad alimentaria causan enfermedades graves a nivel mundial, con una mayor recurrencia en los países en desarrollo. Esto ha resaltado la necesidad de explorar alternativas antimicrobianas efectivas. Este estudio tuvo como objetivo evaluar la actividad

antimicrobiana de extractos etanólicos de *Sambucus canadensis*, *Bidens alba*, *Ricinus communis* y *Salvia microphylla* contra bacterias Gram positivas y Gram negativas mediante pruebas *in vitro* e *in vivo*. Se realizaron análisis fitoquímicos e identificación de grupos funcionales mediante espectroscopia IR. Las pruebas *in vitro* utilizaron técnicas de difusión en disco y microplaca, mientras que las pruebas *in vivo* involucraron *Solanum lycopersicum* para evaluar las cargas microbianas dentro del límite máximo permisible (MAL) de acuerdo con la Norma Oficial Mexicana NOM-093-SSA1-1994. Los análisis fitoquímicos y de IR confirmaron la presencia de flavonoides, terpenos y saponinas, todos los cuales poseen propiedades antimicrobianas conocidas. En general, todos los extractos inhibieron tanto a las bacterias Gram positivas como a las Gram negativas. Cabe destacar que los extractos de *S. canadensis* y *B. alba* exhibieron las mayores zonas de inhibición, las cuales fueron estadísticamente significativas (P<0.05) en comparación con los otros extractos y el control. Los valores de concentración mínima inhibitoria (CMI) variaron de 68.1 a 1000 µg mL^{-1}, y el extracto de *S. canadensis* mostró la mayor inhibición contra *Staphylococcus aureus* y *Klebsiella pneumoniae* con 68.1 y 75.1 µg mL^{-1}, respectivamente. Las pruebas *in vivo* demostraron que todos los extractos redujeron la carga bacteriana de las cepas evaluadas, excepto *Salmonella typhimurium*. Estos hallazgos sugieren que los extractos evaluados podrían ser una alternativa prometedora para los antimicrobianos naturales contra patógenos transmitidos por alimentos que amenazan la seguridad alimentaria y la salud humana.

Palabras clave: antimicrobianos naturales, plantas medicinales, bacterias, resistencia bacteriana.

1 INTRODUCTION

In recent years, diseases caused by foodborne pathogens (FBI) have been on the rise, becoming a global health issue with significant social and economic impacts (Zhao et al. 2017). Each year, millions of people fall ill from eating contaminated food, and thousands die from the same cause (Miyagishima 2015).

Morbidity and mortality are steadily increasing, largely due to the resistance that bacteria have developed against disinfectants and antibiotics, exacerbated by the indiscriminate use of these products (Garayoa et al. 2017). Fruits and vegetables are among the foods with significant safety issues (Marriott et al. 2018). They can be contaminated at any stage of the food supply chain: production, distribution, processing, packaging, or preparation (Rossi et al. 2017).

Current methods to reduce microbiological contamination and mitigate health risks primarily involve the use of chemical substances, such as disinfectants and synthetic preservatives. However, these can have carcinogenic and teratogenic effects due to their residual toxicity (Mola et al. 2016). This situation highlights the urgent need for new and innovative antimicrobial agents.

Plants are a promising source for these new agents, as they have been shown to be effective against multidrug-resistant bacteria, yielding results comparable to those of synthetic compounds (Mostafidi et al. 2020, Soumia et al. 2024). In addition to being biodegradable in the environment, plant-based agents can help prevent issues of microbial resistance that arise from the excessive use of chemically synthetic products (Quinto et al. 2019; Zeng et al. 2012).

Several studies have demonstrated that extracts from various plant species exhibit activity against microorganisms associated with foodborne diseases (Abdallah, 2019; Budiati et al. 2022; Jun-hua et al. 2019). In Mexico, with its rich plant diversity, plants have been utilized as traditional medicine in rural areas for centuries and continue to serve as a primary source of healthcare with positive outcomes (Lucía et al. 2021).

The objective of this study was to assess the antimicrobial activity of ethanolic extracts from Sauco *Sambucus canadensis* L., Amomotzo *Bidens alba* (DC.) L., Higuerilla *Ricinus communis* L. and Mirto *Salvia microphylla* Kunth against microorganisms that impact food safety. The goal was to develop sustainable natural product solutions to combat pathogenic bacteria associated with food contamination and thereby reduce the potential health risks they pose (Khan et al. 2020).

2 MATERIALS AND METHODS

PLANT MATERIAL

The species chosen for this study—*S. canadensis*, *S. microphylla*, *R. communis* and *B. alba*—are used by indigenous communities in the Sierra Negra region of Puebla, Mexico, for treating various ailments. Table 1 provides details on the botanical names, traditional uses, and plant parts utilized. Samples of each plant are preserved in the herbarium of the Botanical Garden of BUAP (Benemérita Universidad Autónoma de Puebla).

Table 1. Medicinal plants traditionally used in the Sierra Negra de Puebla, México.

Botanical name	Family	Local name	Plant part used	Tradicional usage
Sambucus canadensis L.	Adoxaceae	Sauco	Leaves, flowers,	Diarrhea, stomachache, skin infections, anti-inflammatory.
Salvia microphylla Kunth	Lamiaceae	Mirto, cox	Leaves	Stomachache, indigestion skin infections, burns
Bidens alba (DC.) L.	Asteraceae	Amomotzo	Leaves	Indigestion
Ricinus communis L.	Euphorbiaceae	Higuerilla	Leaves, fruits	Indigestion, stomachache

Source: Authors.

EXTRACTS PREPARATION

500 grams of each plant species were collected from locations in the Sierra Negra region of Puebla, Mexico. The leaves were macerated in a 4-liter Erlenmeyer flask using double-filtered 96% ethanol. The extracts were vacuum filtered through Whatman No. 4 paper, and the filtrate was concentrated using a Büchi rotary evaporator (Switzerland, Flawil) under reduced pressure at 35±15 ºC. The ethanolic extracts were further evaporated in a vacuum chamber until completely dry. The yield of the plant extracts was calculated using the equation provided by Benítez-Benítez et al. (2019).

$$\%R = \frac{w_i - w_f}{w_i} * 100$$

Where:
$\%R$ = yield percentage
w_i = initial weight
w_f = final weight after obtaining the extract.

CHEMICAL CHARACTERIZATION OF THE EXTRACTS

For the qualitative identification of various metabolite groups, several standard methods with minor modifications were employed (Abdelkader et al. 2015). These tests included: coumarins (Baljet), triterpenes and steroids (Liebermann-Burchard), saponins (foam and Rosenthaler), tannins (gelatin and ferric chloride), cyanogenic glycosides (Grignard), flavonoids (Shinoda, Pew's, NaOH) and alkaloids (Dragendorff, Mayer, and Wagner). Each test was performed in triplicate. Functional groups were identified using Fourier transform infrared spectroscopy (FTIR) with a Bruker infrared spectrophotometer (Germany, Ettlingen), covering the range of 4000-600 cm^{-1}. The analyses were conducted in the CESAT department at the UPAEP.

TEST MICROORGANISMS

Six strains associated with gastrointestinal diseases that can contaminate food were selected: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 13883), which were obtained from the stock collection of the Department of Biotechnology at the UPAEP. Additionally, two strains, *Salmonella typhimurium* (ATCC 19430) and *Vibrio cholerae* (ATCC 17802), were provided by the Puebla State Health Laboratory.

DETERMINATION OF ANTIMICROBIAL ACTIVITY BY THE DISK DIFFUSION METHOD

The bacterial suspension was prepared by adjusting the inoculum to a concentration of 0.5 on the McFarland scale using a nephelometer or its optical equivalent, corresponding to 10^6 CFU mL^{-1}. Subsequently, 100 µL of the bacterial suspension was spread on solid Mueller-Hinton agar plates (Laboratorios Britania, Argentina) using sterile cotton swabs. Filter paper discs (6 mm diameter, Whatman #1) were impregnated with 40 µL of each plant extract at a concentration of 100 µg mL^{-1}. For negative control, 40 µL of dimethyl sulfoxide (DMSO) was used, and commercial gentamicin discs (10 µg, Laboratorios Britania, Argentina) served as the positive control. The plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zone (DIZ) was measured in millimeters. Inhibition was considered significant for halos greater than 6 mm,

compared to the standard diameter of the sensi-discs used (Zeng et al. 2012). The tests were performed in triplicate and the results of the inhibition zones were recorded in millimeters (Table 2).

Table 2. Qualitative analysis of phytochemicals in ethanolic extracts.

	Alkaloids			**Flavonoids**			**Saponins**		**Coumarins**	**Triterpenes and/or steroids**	**Glycosides cyanogenic**	**Tannins**	
Extract	M	D	W	N	S	P	E	R	B	LB	G	GL	$FeCl_3$
R. communis	++	+++	++	-	+	+	++	+++	++	++	-	++	++
B. alba	++	++	++	-	+	+	++	+++	-	-	-	++	+
S. microphylla	++	++	++	-	+	+	++	++	++	++	-	++	+
S. canadensis	++	++	+++	-	+++	++	++	++	-	-	-	++	++

Intensity: heavy presence +++, medium presence++, slight presence+, not detected –

*Alkaloids: M = Mayer test , D = Drangendorff test W = Wagner test
Flavonoids: S = Shinoda test N:NAOH P: Pew's
Saponins: E = Foam test, R= Rosenthaler test
Coumarins: B = Baljet test
Triterpenes and/or steroids: LB = Lieberman-Bouchard test
Glycosides cyanogenic: G= Grignard test
Tannins: Gelatin reagent = GL, ferric chloride=$FeCl_3$

Source: Authors.

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) BY MICROPLATE

After assessing the bacterial activity of the plant extracts, the Minimum Inhibitory Concentration (MIC) was determined using the microdilution method in a 96-well microplate. An aliquot of 150 µL of culture medium was added to each well, along with 20 µL of each bacterial strain adjusted to 0.5 McFarland scale and 30 µL of each ethanolic extract. Serial dilutions of the extracts were prepared in concentration ranges from 125 to 1000 µg mL^{-1}. The microplates were incubated at 37ºC for 24 hours, and absorbance was measured with an ELISA microplate reader at 560 nm. All experiments were performed in triplicate. The MIC values were determined as the concentration where the optical density (O.D.) matched that of the initial inoculum after 24 hours of incubation under standardized conditions.

IN VIVO TESTS IN *SOLANUM LYCOPERSICUM*

The experimental units were tomatoes (*Solanum lycopersicum*), selected due to their significant safety issues related to pathogenic microorganisms. The samples were collected from two local supermarkets in the State of Puebla. The bacteria used were previously cultured in nutrient medium (Bioxon, Mexico City, Mexico) for 24 hours at 37 ºC until reaching an optical density (O.D.) equivalent of 0.5 McFarland. Concentrations of 500 and 1000 µg mL^{-1} were selected based

on the inhibition results obtained from the microplate tests. Inoculation of the tomatoes with the pathogenic bacteria was performed by immersion, following the methods described by Tornuk et al. (2011). The extracts were then applied by spraying (5 mL), and after 24 hours, the application was repeated using the same method (Luna-Guevara et al. 2015). Three treatments were conducted for each of the six pathogenic bacteria and each of the four extracts: T1, tomato with bacteria; T2, tomato with extract and bacteria; T3, tomato without bacteria and without extract; with three replicates for each treatment.

Microbiological safety analyses were conducted 24 hours after the previous procedure. The entire fruit was homogenized in a previously sterilized blender, and 1 mL of this mixture was transferred to 9 mL of sterile peptone water, with subsequent serial dilutions ranging from 10^{-2} to 10^{-4}. The number of colony-forming units (CFU) per gram was quantitatively assessed. Following this, quality tests for total mesophiles and enterobacteria were performed according to the Official Mexican Standard NOM-093-SSA1-1994.

STATISTICAL ANALYSIS

ANOVA and Tukey's post hoc tests were used to determine differences between treatments. Differences were considered significant with a $P<0.05$. The analyses were conducted using the Minitab statistical software (licensed for UPAEP).

3 RESULTS AND DISCUSSION

EXTRACT'S YIELD AND CHARACTERIZATION

Currently, products derived from natural sources are garnering significant interest due to their lower environmental impact and enhanced safety for human health (Quinto et al. 2019; Walid et al. 2024). In order to assess the antimicrobial properties of *S. canadensis*, *B. alba*, *R. communis* and *S. microphylla*, ethanolic extracts were obtained. The plants used in this study were selected based on their traditional medicinal use in indigenous communities in Mexico for treating various diseases. The extracts from these plants were found to contain compounds such as alkaloids, saponins, tannins and flavonoids, which contribute to their diverse antimicrobial activities.

The yields of the ethanolic extracts were as follows: *R. communis* 7.23%, *S. microphylla* 6.42%, *B. alba* 7.18% and *S. canadensis* 6.11%. In the phytochemical tests, most extracts primarily contained alkaloids among the evaluated components. *R. communis*, *B. alba* and *S. microphylla* also tested positive for saponins, while *S. canadensis* was noted for the presence of flavonoids (Table 2).

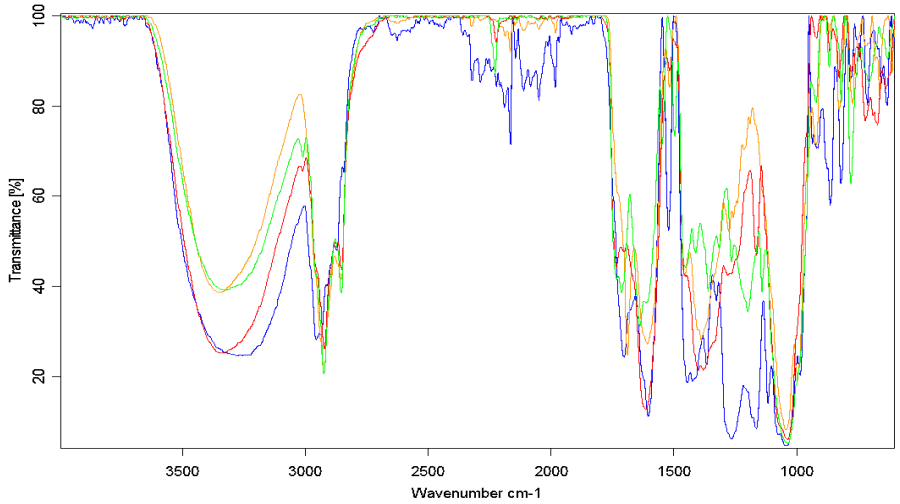
Regarding the FTIR spectra, the *R. communis* extract displayed a C-H bond stretching signal at 2853-2924 cm^{-1} and a signal at 1733-1637 cm^{-1}, indicative of the C=O carbonyl group.

Additionally, C-O signals were observed at 1034 cm^{-1}, which are characteristic of alkaloids. The *B. alba* spectrum showed a broad O-H strain signal at 3343.26 cm^{-1}, a C-H stretching signal at 2923.28 cm^{-1}, C=O carbonyl group signals at 1614.06 cm^{-1}, and an intense C-O signal at 1036 cm^{-1}, also associated with alkaloids. The spectrum of *S. microphylla* revealed an O-H stretching signal at 3273-2870 cm^{-1}, an intense C=O signal at 1602-1702 cm^{-1}, and C-C signals at 1043, 1173, and 1326 cm^{-1}, which are characteristic of the carbonyl group.

The absorption of the ketone group results in multiple bands in the IR spectrum. Aliphatic ketones typically absorb in the range of 1230 cm^{-1} to 1100 cm^{-1}. In our samples, the signals at 1326 cm^{-1} and 1173 cm^{-1} were identified as vibrational modes of stretching and bending corresponding to the ketone group, which is characterized by narrow and weak peaks.

The spectrum of *S. canadensis* exhibited an O-H stretching signal at 3359.01 cm^{-1}, a C-H stretching signal at 2924.69 cm^{-1}, and a C-H bond bending signal at 1401.71 cm^{-1}, which are indicative of flavonoids. Figure 1 displays the four infrared spectra with their key signals highlighted. The broad absorption for O-H groups, ranging from 3273.34 to 3350.01 cm^{-1}, suggests the potential formation of intermolecular hydrogen bonds. The C-H stretching signal within the range of 2853.52 to 2955 cm^{-1} indicates the presence of C=C double bonds between 1400 and 1600 cm^{-1}. Additionally, other bands characteristic of carbonyl groups was observed in the regions of 1600 cm^{-1} and 1734 cm^{-1}, respectively.

Figure 1. FTIR spectra of four extracts in the range of 4000 to 600 cm^{-1}: *S. canadensis*, yellow line; *R. communis*, green line; *B. alba*, red line; *S. macrophylla*, blue line.



Source: Authors.

Tannins are known to inhibit extracellular microbial enzymes (Martínez-Frances et al. 2017). Saponins, on the other hand, form complexes with sterols in cell membranes, creating large pores and altering membrane permeability. This leads to cell lysis by rupturing bacterial membranes

(Ramírez-Díaz et al. 2012). Due to this mechanism, saponins isolated from leaves, fruits, seeds or stems are commonly used in soaps and antimicrobial products (Verma et al., 2014)

Alkaloids primarily function to protect plants from environmental microorganisms, they exhibit inhibitory effects on pathogenic microorganisms by damaging microbial DNA, halting protein synthesis and inhibiting enzymes involved in carbohydrate metabolism, which induces cellular apoptosis. (Rodríguez-Sauceda, 2011).

Flavonoids possess antifungal, antiviral and antibacterial activities. Some isolated flavonoids, such as quercetin and rutin, inhibit DNA and RNA synthesis and cause damage to bacterial cell membranes (Wang et al. 2013)

According to Ng et al. (2014) the presence of these compounds in the evaluated extracts could account for the antimicrobial activity observed in this study. Additionally, it has been suggested that the synergy of compounds present in crude extracts may produce more effective results than the individual components alone (Mehmood and Murtaza 2018).

DIZ TESTS

To evaluate the capacity of the extracts to inhibit or halt the growth of pathogenic bacteria, Disc Inhibition Zones (DIZ) tests were performed on *S. aureus, E. faecalis, E. coli, K. pneumoniae, S. typhimurium* and *V. cholerae's* growth. All plant extracts demonstrated antimicrobial activity using the disk diffusion method. The results for the diameters of the inhibition zones (DIZ) are presented in Table 3. Different concentrations of *S. canadensis* extracts exhibited an inhibitory effect against *S. aureus*, with an inhibition diameter of 12 mm; however, *V. cholerae* showed minimal sensitivity to this extract, with an inhibition diameter of 7.7 mm. The *R. communis* extract produced an inhibition zone greater than 9 mm against *S. aureus* and *K. pneumoniae*. The extracts of *S. microphylla* and *B. alba* exhibited the highest activity, with inhibition halos greater than 10 mm against *S. aureus* and *K. pneumoniae*.

Table 3. Antibacterial activity in Diameter of Inhibition Zone (DIZ) of extracts against test microorganisms. Source: Authors.

	DIZ (mm) ± S.D					
Extract	**Pathogens**					
	K. pneumonie	*E. coli*	*S. aureus*	*V. chlorae*	*S. typhi*	*E. faecalis*
S. canadensis	10,7±0,6^{I}	9,3±0,5^{I}	12,7±1,5^{S}	7,7±0,6^{R}	9,7±1,5^{I}	9,3±0,6^{I}
R. communis	9,3±1,0^{I}	8,1±1,0^{R}	9,0±1,0^{I}	7,3±0,6^{R}	8,3±0,6^{R}	8,0±1,0^{R}
S. microphylla	10,0±1,0^{I}	8,0±1,0^{R}	12,3±0,6^{S}	6,3±0,6^{R}	7,6±0,5^{R}	8,6±1,1^{R}
B. alba	10,3±0,6^{I}	9,3±0,6^{I}	10,3±0,6^{I}	7,3±0,6^{R}	7,7±0,6^{R}	9,3±0,6^{I}
DMSO	7,0±1,0^{R}	6,3±0,6^{R}	6,3±1,0^{R}	-	-	-
Gentamicin	12,6±0,57^{I}	10,6±1,5^{I}	13,3±0,5^{S}	10,6±0,57^{I}	11,3±1,1^{S}	11,0±1,0^{S}

*Resistant (R)
Intermediate (I)
Susceptible (S)

In the DIZ, all extracts showed inhibition zones ≤8 mm against the evaluated bacteria, except for *V. cholerae* and *S. typhimurium*. The ethanolic extract of *S. canadensis* leaves exhibited the largest inhibition zones, with maximum diameters of 12.7 mm against *S. aureus* and 10.7 mm against *K. pneumoniae*. Other studies have reported similar findings for related species: Rodino et al. (2015) found that *Sambucus nigra* produced inhibition halos greater than 10 mm against *E. faecalis*; Benevides et al. (2017) observed significant inhibitory effects of *Sambucus australis* against *Salmonella typhimurium* and *K. pneumoniae*; and Goud and Prasad (2020) reported inhibition zones of 12 mm for *S. nigra* against *E. coli*. These studies support the antimicrobial efficacy of the *Sambucus* species.

The *R. communis* extract displayed inhibition zones ranging from 8 to 9 mm against *E. coli*, *S. aureus* and *K. pneumoniae*. These results differ from those reported by Verma et al. (2014), who observed inhibition zones ≤20 mm for some of the same bacteria, such as *E. coli* and *S. aureus*. However, the study by Verma et al. used a concentration of 200 mg mL^{-1}, which is significantly higher than the 100 µg mL^{-1} used in our study. Similarly, Suurbaar et al. (2017) reported inhibition halos of 20, 21 and 24 mm for *E. coli*, *S. aureus* and *K. pneumoniae*, respectively, which can be attributed to the high concentration of 50 mg mL^{-1} tested in their study.

The *B. alba* extract exhibited the largest inhibition zones, measuring 10.3 mm against *S. aureus* and *K. pneumoniae*. These findings align with those of Chavasco et al. (2014), who reported that *Bidens pilosa* showed antimicrobial effects with inhibition zones of 22 mm against *S. aureus*, 14 mm against *E. faecalis* and 12 mm against *E. coli*. Additionally, Chiavari et al. (2020), have noted significant antibacterial and antifungal activities of *B. alba* extract.

On the other hand, *S. microphylla* showed significant inhibition against *S. aureus* (12.1 mm) and *K. pneumoniae* (9 mm). This is consistent with reports from other studies indicating that *Salvia* species possess significant antibacterial activity against both Gram-positive and Gram-negative bacteria (Abdelkader et al. 2015). It has been suggested that the antimicrobial activity of *Salvia* spp. extracts has the potential to impact the prevalence of both susceptible and resistant foodborne microorganisms. Consequently, extracts and essential oils from this species could serve as natural antimicrobial alternatives (Sharifi-Rad et al. 2018).

MINIMUM INHIBITORY CONCENTRATION (MIC)

To assess the effectiveness of the ethanolic extracts, the Minimum Inhibitory Concentration (MIC) test was performed on the different bacterial strains using microdilution with 96-well plates. The most susceptible strains were *S. aureus*, *K. pneumoniae* and *E. coli*, with MIC values of 75.8,

65.1 and 100 µg mL^{-1}, respectively. Conversely, *V. cholerae* and *S. typhimurium* were the most resistant to the extracts, with MIC values exceeding 1000 µg mL^{-1} (Table 4).

Table 4. Minimum Inhibitory Concentration (MIC)of extracts against test microorganisms.

Extract / MIC (µg mL^{-1}) per species	***K. Pneumoniae***	***E. coli***	***S. Aureus***	***E. faecalis***	***S. typhimarium***	***V. chlorae***
S. canadensis	68.1	115.18	75.6	>450	<1000	<500
R. communis	200	200	<1000	>300	<1000	>250
S. microphylla	>200	>100	>400	>400	>1000	>500
B. alba	<1000	>300	100	100	<1000	>1000

Source: Authors.

Regarding the *S. canadensis* extract, it demonstrated an inhibitory effect against *K. pneumoniae* (MIC = 68.1 µg mL^{-1}) and *S. aureus* (MIC = 75.6 µg mL^{-1}). These findings are consistent with reports by Hearst et al. (2010) and Holetz et al. (2002), which also highlighted the efficacy of *S. canadensis* against *S. aureus*. Conversely (Benevides-Bahiense et al. (2017) found that *Sambucus australis* exhibited antibacterial activity against *S. typhimurium* (250 µg mL^{-1}) and *K. pneumoniae* (250 µg mL^{-1}), which differs from our results with *S. canadensis*.

Regarding *R. communis*, it showed inhibition effects greater than 1000 µg mL^{-1}, which contrasts with the findings of Suurbaar et al. (2017), who reported values below 1000 µg mL^{-1} for *E. coli* and *S. aureus*. Additionally, El-Naggar et al. (2019) reported that metabolites from *R. communis* exhibited strong activity against *S. aureus*, *E. coli* and *K. pneumoniae*, although they noted only moderate activity against other strains.

Although the antimicrobial activity of the metabolites from the species was not evaluated in this study, Mehmood and Murtaza (2018) suggest that the synergy among these compounds could explain the results observed. The *B. alba* extract showed significant activity against *S. aureus*, *E. faecalis* and *E. coli*. In contrast, Falowo et al. (2016) reported lower inhibition values for *Bidens pilosa* against these strains. This discrepancy may be attributed to the specific metabolites present in the species, such as flavonoids, which, according to Connelly (2009), are common to other subspecies, including *B. alba*.

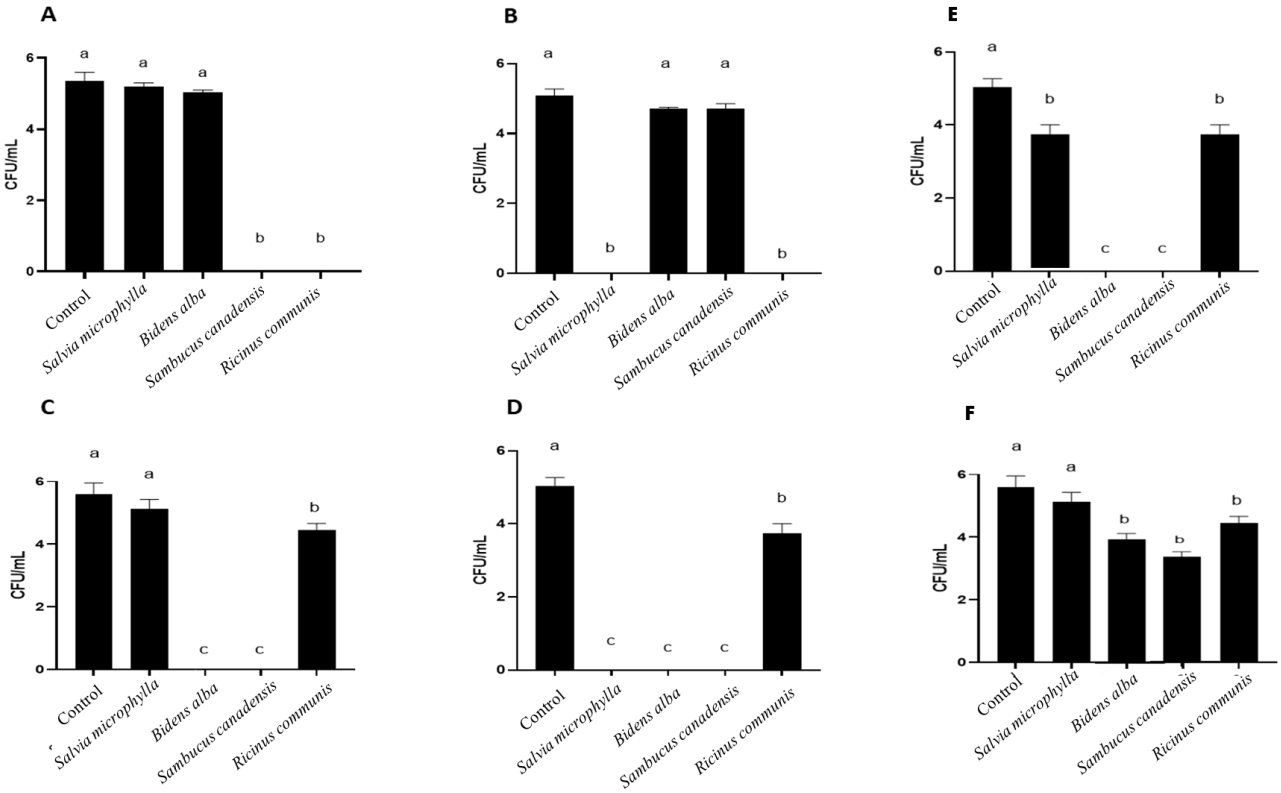
Regarding the *S. microphylla* extract, the MIC for *S. aureus* found in this study was lower than the 7.81 mg mL-1 reported by Fraternale et al. (2012) noted that *S. microphylla* shows better activity against Gram-positive bacteria compared to Gram-negative bacteria such as *E. coli*; however, in this study, activity was observed against both types of bacteria. Naz and Bano (2012) and Weerakkody et al. (2010) suggest that Gram-positive bacteria are generally more sensitive to plant extracts than Gram-negative bacteria. Despite this, *S. canadensis* exhibited lower inhibition

values for Gram-negative bacteria in this study, which may be attributed to the specific metabolites present in the species.

IN VIVO TESTS IN *SOLANUM LYCOPERSICUM*

In the *in vivo* tests, most of the strains evaluated showed a significant reduction in bacterial load with at least two of the plant extracts compared to the control. In some cases, the bacterial load was completely reduced to meet the maximum permissible limits established by the Official Mexican Standard NOM-093-SSA1-1994. However, for the *S. typhimurium* strain, although significant differences were observed compared to the control, the bacterial load was not fully reduced. The results are illustrated in Figure 2.

Figure 2. *In vivo* test antimicrobial activity using *Solanum lycopersicum*. The CFU mL-1 values for each bacterium are shown with the four extracts evaluated at 500 µg mL-1: A) *S. aureus*, B) *E. faecalis*, C) *E. coli*, D) *K. pneumoniae*, E) *V. cholerae*, and F) *S. typhimurium*. Different letters indicate statistically significant differences (P<0.05).



Source: Authors.

Few studies have evaluated the effectiveness of plant extracts in *in vivo* tests, and some focus only on their effectiveness against pathogenic fungi (Luna-Guevara et al. 2015). This study demonstrated that all four plant extracts were effective in reducing bacterial load, in accordance with the maximum permissible limits of the Mexican Official Standard NOM-093-SSA1-1994

(Figure 2). Notably, the extracts also showed efficacy against bacteria that are indicators of microbiological quality or shelf life of foods, such as *E. coli* (Falowo et al. (2016)

FINAL REMARKS

The use of natural products to reduce bacterial load could be of great interest to the food industry, especially in countries like Mexico that face significant safety issues and where many people are affected by consuming contaminated food. The results of this study align with those reported by Tornuk et al. (2011) Suurbaar et al. (2017) demonstrating that plants can be effectively used in the food industry as antibacterials due to their activity against both Gram-negative and Gram-positive bacteria.

Similarly, according to Mostafidi et al. (2020) and Gadoum et al. (2024), plant extracts are an important source for discovering new antimicrobials due to their extensive chemical diversity. Therefore, it is crucial to promote research on the use of plants as effective alternatives for combating bacteria that affect food.

4 CONCLUSION

This study demonstrated that plant extracts from *R. communis*, *S. microphylla*, *S. canadensis* and *B. alba*, all used in traditional medicine, exhibit antimicrobial effects against pathogenic bacteria. These results are associated with the presence of the metabolites identified in this research. Both *in vitro* and *in vivo* tests revealed that the extracts were capable of inhibiting bacterial growth in the evaluated strains. Therefore, it can be concluded that plant extracts used as traditional medicine in indigenous communities of the Sierra Negra de Puebla, Mexico, are effective against bacteria of nutritional interest and may serve as potential alternatives for combating foodborne pathogens.

Nevertheless, the scope of this study is confined to the ethanolic extracts of *S. canadensis*, *S. microphylla*, *R. communis*, and *B. alba*, tested against the bacterial strains *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Salmonella typhimurium* (ATCC 19430), and *Vibrio cholerae* (ATCC 17802). Consequently, the findings cannot be generalized to other genera or species. Further studies are warranted to explore broader antimicrobial applications.

As final recommendations, it is crucial to undertake studies aimed at preserving and recovering traditional medicinal knowledge within indigenous communities, which possess a rich diversity of plant species. Identifying the chemical composition of plant extracts through gas chromatography-mass spectrometry (GC-MS) analysis is essential for understanding their bioactive

components. Scientific investigations are necessary to validate the therapeutic potential of these plants, including assessments of cytotoxic and genotoxic activity, particularly given their widespread consumption among the population. Furthermore, research involving diverse microbial strains is highly relevant to confirm and expand upon the potential effects of the plant extracts evaluated in this study.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

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