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Bactericidal Activity of *Larrea tridentata* Hydroalcoholic Extract against Phytopathogenic Bacteria

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Citation: Morales-Ubaldo, A.L.; Rivero-Perez, N.; Avila-Ramos, F.; Aquino-Torres, E.; Prieto-Méndez, J.; Hetta, H.F.; El-Saber Batiha, G.; Zaragoza-Bastida, A. Bactericidal Activity of *Larrea tridentata* Hydroalcoholic Extract against Phytopathogenic Bacteria. *Agronomy* **2021**, *11*, 957. https://doi.org/10.3390/agronomy11050957

Academic Editor: Mukesh Dubey

Received: 15 April 2021
Accepted: 10 May 2021
Published: 12 May 2021

Abstract: Due to the emergence of bacterial resistance in phytopathogenic microorganisms, it is necessary to search for new treatment alternatives for these pathogens. Natural extracts are a potential source of bioactive compounds that can act against such bacterial strains. The antibacterial activity of *Larrea tridentata* against bacteria with public health importance has been documented; however, few reports cover pathogens associated with the agricultural sector. The aim of the present study was to evaluate the antibacterial activity of *Larrea tridentata* hydroalcoholic extract (LTHE) and fractions against phytopathogenic bacteria. LTHE was obtained by the maceration technique and then subjected to bipartition using solvents of different polarities. *Clavibacter michiganensis* sbsp. *michiganensis*, *Pseudomonas syringae*, and *Xanthomonas campestris* strains were used, and their antibiotic sensitivity was determined. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of LTHE and its fractions (aqueous: LTAq-F; organic: LTEtOAc-F) were determined. LTHE and its organic fraction showed bactericidal activity against the three bacteria, showing better activity against *X. campestris*, exhibiting an MIC of 0.39 mg/mL and an MBC of 0.78 mg/mL. The results show that LTHE and its organic fraction have bactericidal activity in vitro against *Clavibacter michiganensis* sbsp. *michiganensis*, *Pseudomonas syringae*, and *Xanthomonas campestris*.

Keywords: *Larrea tridentata*; hydroalcoholic extract; organic fraction; bactericidal activity; phytopathogenic bacteria

1. Introduction

The agricultural activities around the world are the main food suppliers, hence its great relevance. Over the years, this sector has undergone significant changes, such as adaptions to new demands by society, changes in soil conditions and climate, these changes have triggering fires, droughts, de-creased crop yield, and the emergence or reemergence of pests and diseases [1,2].

Production of tomato (*Solanum lycopersicum* L.), one of the most consumed vegetables, has been limited by several factors, including management practices, pests, and

diseases [3,4]. Some reports state that more than 60 pathogens, including viruses, nematodes, fungi, and bacteria, can infect tomato crops, which results in severe problems that affect global production [5]. Among the most important diseases are bacterial canker, bacterial speck, and bacterial spot, whose etiological agents are *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae*, and *Xanthomonas campestris*, respectively [4,6,7].

Controlling phytopathogenic bacteria currently represents a challenge due to the lack of effective antibacterial agents, since these microorganisms have developed resistance to the active ingredients, in addition to the environmental damage resulting from their application. This situation has impeded the treatment and control of several diseases, and in some cases, there are no chemical or biological treatments available with which to control them, as in the case of *Xanthomonas campestris* [8–11].

Due to the aforementioned problems, new treatment alternatives are being sought. Today, biocontrol is sociologically, commercially, and environmentally accepted as a legitimate tool for crop protection [12]. In this respect, medicinal plants, through the use of extracts, have attracted attention in the field of plant disease control, since they are considered biopesticides. Among the main advantages of botanical agents are that they are less toxic, biodegrade rapidly, are targeted to specific pests, and exhibit specific modes of action, thus maintaining the ecological balance [13,14].

Larrea tridentata is a perennial shrub with wide distribution (deserts in Mexico and USA) that survives in hostile environments and is characterized by resistance to pests and infections [15,16]. Is a botanical species well known in Mexican and American traditional medicine for its effectiveness against infertility, rheumatism, arthritis, diabetes, renal afflictions, pain, and inflammation [17,18].

Several studies have reported the pharmacological properties of *Larrea tridentata*, such as antioxidant [19–25], antitumoral [23], neuroprotective [26,27], regenerative [28], hepatoprotective [29–31], nephroprotective [32,33], antiviral [34], antifungal (mainly against fungi associated with agricultural crops, including tomatoes) [22,35–38], antiparasitic [39], and antibacterial (against Gram-positive and Gram-negative bacteria and Mycobacteria) effects [40–49]. However, there are no reports on the antibacterial activity of this plant against pathogens that affect agricultural production, so the aim of the present study was to evaluate the antibacterial activity of hydroalcoholic extract and fractions of *Larrea tridentata* against phytopathogenic bacteria.

2. Materials and Methods

2.1. Plant Material

Aerial parts from *Larrea tridentata* plants were collected in the municipality of Matehuala, San Luis Potosí, Mexico (23°38′47′′ *n* 100°30′40′′ O). For plant identification, the herbarium of the National Autonomous University of Mexico (UNAM) was consulted, and the plant was verified as *Larrea tridentata* (IBUNAM: MEXU: 1249920). Fresh material was dried at room temperature in the dark.

2.2. Preparation of Hydroalcoholic Extract

Hydroalcoholic extract was prepared according to the methodology described by Rivero-Perez et al. (2019) [50]. Dried aerial parts from *L. tridentata* (1500 g) were subjected to an extraction process through the maceration technique using a hydroalcoholic solution of ethanol:water (70:30 *v*/*v*) at room temperature for 24 h. Subsequently, it was filtered using Whatman filter paper (Whatman® 42). After filtration, the solvent was eliminated using a rotary evaporator to obtain a semisolid extract (Büchi R-300, Flawil, Switzerland), which, finally, was lyophilized (LABCONCO®) and stored at 4 °C until antibacterial evaluation.

2.3. Fractionation of Larrea Tridentata Hydroalcoholic Extract

Hydroalcoholic extract was fractionated according to the methodology described by González-Alamilla et al. (2019), and Olmedo-Juárez et al. (2019), described below [51,52].

L. tridentata hydroalcoholic extract (LTHE) was processed for bipartition via liquid-liquid chromatography, using water: ethyl acetate (1:3) solvent (Merck, Darmstadt, Germany). Two fractions were obtained, aqueous fraction (LTAq-F) and organic fraction (LTEtOAc-F). Solvent in both fractions was eliminated by low-pressure distillation (Büchi R-300, Flawil, Switzerland). Both fractions were evaluated with a pharmacological antibacterial test.

2.4. Bacterial Strains and Culture Conditions

The strains used were *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae*, and *Xanthomonas campestris*, which were obtained from AG laboratory in Celaya Guanajuato, México.

Bacterial strains were reactivated from cryopreservation in Müller–Hinton agar (BD Bioxon, Heidelberg, Germany) with the simple strain technique to obtain isolated colonies. Gram staining was performed to corroborate their morphology and purity. One colony of each strain was inoculated in nutritive broth (BD Bioxon) and incubated for 24 h at 26 °C.

2.5. Antibiotic Sensitivity Test

Antibiotic sensitivity was determined using the disk diffusion method in Müller–Hinton agar (BD Bioxon, Heidelberg, Germany), according to the methodology described by Rangel-López et al. (2020) [53].

For this process, 100 µL of each bacterium previously adjusted to a 0.5 McFarland standard (Remel, R20421, Lenexa, KS, USA) was inoculated and distributed on petri plates, and each plate was allowed to dry for 15 min. Once this period elapsed, multidiscs (PT-35, Mexico City, Mexico) were placed on the plate and incubated at 26 °C for 24 h. After the incubation period, growth inhibition halos were measured and compared with measures established by the Clinical and Laboratory Standards Institute (CLSI) [54].

Antibacterial active ingredients used were amikacin (30 µg), ampicillin (10 µg), carbenicillin (100 µg), cephalothin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), clindamycin (30 µg), chloramphenicol (30 µg), dicloxacillin (1 µg), erythromycin (15 µg), gentamicin (10 µg), netilmicin (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), penicillin (10 U), sulfamethoxazole/trimethoprim (25 µg), tetracycline (30 µg), and vancomycin (30 µg).

2.6. Antibacterial Activity

The antibacterial activity of the *L. tridentata* hydroalcoholic extract and fractions was determined through the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in accordance with the CLSI guidelines, and as described by Zaragoza-Bastida et al. (2020) [54,55].

2.6.1. Minimal Inhibitory Concentration (MIC)

Microdilution technique was used to determine MIC, and different concentrations were evaluated (200–3.12 mg/mL for LTHE and 6.25–0.39 mg/mL for LTAq-F and LTEtOAc-F). Every concentration was prepared with nutritive broth, except for the organic fraction, which was solubilized in Dimethyl Sulfoxide Solvent (DMSO) (15%).

Into a sterile 96-well plate, 100 µL of each concentration were added along with 10 µL of bacterial cell suspension previously adjusted to a 0.5 McFarland standard (Remel, R20421, Lenexa, KS, USA). Plates were incubated at 26 °C during 24 h. Kanamycin (AppliChem 4K10421, Darmstadt, Germany) (16 to 0.125 µg/mL) and nutritive broth, were used as positive and negative controls, respectively. Treatments were evaluated by triplicate.

After incubation, 20 µL of a 0.04% (*w/v*) p-iodonitrotetrazolium (Sigma-Aldrich I8377, St. Louis, MO, USA.) solution was added into each well, and the plates were incubated for 30 min. The MIC was determined by the concentration at which the solution turned to a pinkish color.

2.6.2. Minimal Bactericidal Concentration (MBC)

After incubation and prior to adding p-iodonitrotetrazolium, 5 µL from each well was inoculated in Müller–Hinton agar (BD Bioxon) and incubated at 26 °C for 24 h. The MBC was considered as the lowest concentration where no visible growth of bacteria was observed on the plate.

In order to determine if the evaluated treatments had bactericidal or bacteriostatic effects, the ratio of MBC/MIC was determined. A bacteriostatic effect was considered when the ratio was greater than 4, and a bactericidal effect when values less than or equal to 4 were obtained [51].

2.7. Statistical Analysis

The MIC and MBC results were normalized using log10 and were analyzed by a completely randomized design through ANOVA using the general linear model (GLM). Differences among the means were assessed by Tukey's multiple comparison statistical analysis at the $p = 0.05$ level of significance using the SAS program, version 9.0.

3. Results

3.1. Test of Antibiotic Sensitivity

The results for antibiotic sensitivity show that *Clavibacter michiganensis* subsp. *michiganensis* was resistant to 13 antimicrobials and had intermediate resistance to dicloxacillin, clindamycin, and norfloxacin, making it the most resistant of all the strains under study, followed by *X. campestris*, which was resistant to nine antibiotics and had intermediate resistance to amikacin, and *P. syringae*, which was resistant to eight antibiotics and had intermediate resistance to carbenicillin (Table 1).

Table 1. Inhibition halos (mm) and antibiotic sensitivity of *C. michiganensis* sbsp. *michiganensis*, *P. syringae*, and *X. campestris*.

Antimicrobial	Bacteria		
	C. michiganensis* subsp. *Michiganensis	***P. syringae***	***X. campestris***
Amikacin	10 (R)	20 (S)	15 (I)
Ampicillin	7 (R)	15 (S)	7 (R)
Carbenicillin	7 (R)	16 (I)	22(S)
Cephalothin	7 (R)	11 (R)	7 (R)
Cefotaxime	7 (R)	7 (R)	7 (R)
Ciprofloxacin	30 (S)	30 (S)	7 (R)
Clindamycin	15 (I)	7 (R)	34 (S)
Chloramphenicol	8 (R)	31 (S)	30 (S)
Dicloxacillin	12 (I)	7 (R)	7 (R)
Erythromycin	7 (R)	7 (R)	23 (S)
Gentamicin	7 (R)	21 (S)	12 (R)
Netilmicin	8 (R)	20 (S)	10 (R)
Nitrofurantoin	9 (R)	22 (S)	8 (R)
Norfloxacin	15 (I)	20 (S)	7 (R)
Penicillin	7 (R)	7 (R)	22 (S)
Sulfamethoxazole/ trimethoprim	7 (R)	7 (R)	17 (S)
Tetracycline	20 (S)	20 (S)	20 (S)
Vancomycin	7 (R)	7 (R)	21 (S)

R, resistant; I, intermediate; S, sensitive.

3.2. Minimal Inhibitory Concentration (MIC)

As can be seen in Table 2, *L. tridentata* hydroalcoholic extract inhibited the growth of both Gram-positive and Gram-negative bacteria, and *X. campestris* was the most sensitive bacterium ($p = 0.0001$, MIC = 0.39 mg/mL) compared with *C. michiganensis* subsp.

michiganensis and *P. syringae* (MIC = 6.25 mg/mL). LTEtOAc-F was the most active fraction, showing the best (p = 0.0001) MIC (0.39 mg/mL) against *X. campestris*, followed by *C. michiganensis* sbsp. *michiganensis* and *P. syringae* (MIC = 3.12 mg/mL). LTAq-F only showed activity against two of the strains at 6.25 mg/mL.

Table 2. Minimal inhibitory concentration (mg/mL) of hydroalcoholic extract and fractions obtained from *Larrea tridentata*.

Evaluated Treatment	Bacteria			
	C. michiganensis* sbsp. *Michiganensis	***P. syringae***	***X. campestris***	***p*-Value**
LTHE	6.25 ^{cB}	6.25 ^{cB}	0.39 ^{bA}	0.0001
LTAq-F	6.25 ^{cA}	6.25 ^{cA}	NA	0.0001
LTEtOAc-F	3.12 ^{bB}	3.12 ^{bB}	0.39 ^{bA}	0.0001
Kanamycin	0.25 ^{a*}	0.25 ^{a*}	8 ^{a*}	
p-value	0.0001	0.0001	0.0001	

NA, no activity; LTHE, *L. tridentata* hydroalcoholic extract; LTAq-F, aqueous fraction; LTEtOAc-F, organic fraction. * Values are expressed in µg/mL. ^{a,b,c} Different letters in columns indicate significant statistical differences ($p \leq 0.05$) between treatments, ^{A,B} Different letters in rows indicate significant statistical differences ($p \leq 0.05$) between bacteria.

3.3. Minimal Bactericidal Concentration (MBC)

LTHE showed bactericidal activity against the three strains under study; the best activity (p = 0.0001) was observed for *X. campestris*, with an MBC of 0.78 mg/mL. LTEtOAc-F showed effects against the three strains, with a better MBC (0.0001) for *X. campestris* (0.78 mg/mL and 6.25 mg/mL for the others). LTAq-F did not show an MBC against any bacteria. These results are shown in Table 3.

Table 3. Minimal bactericidal concentrations (mg/mL) of extract and fractions obtained from *Larrea tridentata*.

Evaluated Treatment	Bacteria			
	C. nichiganensis* sbsp. *Michiganensis	***P. syringae***	***X. campestris***	***p*-Value**
LTHE	12.5 ^{cB}	12.5 ^{cB}	0.78 ^{bA}	0.0001
LTAq-F	NA	NA	NA	
LTEtOAc-F	6.25 ^{bB}	6.25 ^{bB}	0.78 ^{bA}	0.0001
Kanamycin	0.5 ^{a*}	0.5 ^{a*}	16 ^{a*}	
p-value	0.0001	0.0001	0.0001	

NA, no activity; LTHE, *L. tridentata* hydroalcoholic extract; LTAq-F, aqueous fraction; LTEtOAc-F, organic fraction. * Values are expressed in µg/mL. ^{a,b,c} Different letters in columns indicate significant statistical differences ($p \leq 0.05$) between treatments. ^{A,B} Different letters in rows indicate significant statistical differences ($p \leq 0.05$) between bacteria.

Calculating the MBC/MIC ratio allowed us to determine that LTHE and LTEtOAc-F had bactericidal effects against *C. michiganensis* sbsp. *michiganensis*, *P. syringae*, and *X. campestris*, since the ratio was 2 for all strains.

4. Discussion

In the present study, an antibiotic sensitivity test was performed with *C. michiganensis* subsp *michiganensis*, *P. syringae*, and *X. campestris* strains. The obtained data show that *C. michiganensis* subsp. *michiganensis* was resistant to 13 antimicrobials (amikacin, ampicillin, carbenicillin, cephalothin, cefotaxime, chloramphenicol, erythromycin, gentamicin, netilmicin, nitrofurantoin, penicillin sulfamethoxazole/trimethoprim, and vancomycin); *X. campestris* was resistant to 9 (ampicillin, cephalothin, cefotaxime, ciprofloxacin, dicloxacillin, gentamicin, netilmicin, nitrofurantoin, and norfloxacin); and *P. syringae* was

resistant to 8 (cephalothin, cefotaxime, clindamycin, dicloxacillin, erythromycin, penicillin, sulfamethoxazole/trimethoprim, and vancomycin).

According to these results, all strains were classified as multidrug-resistant. López-Pueyo et al. (2011) noted that a bacterial strain can be considered to be in this category when it presents resistance to more than one family of antibiotics, such as *C. michiganensis* subsp *michiganensis*. Specifically for Gram-negative bacilli, it must be resistant to three or more families of antibiotics to which they are usually sensitive, such as beta-lactams, carbapenems, aminoglycosides, or quinolones, which was shown by *P. syringae* and *X. campestris* in the present study [56].

Antimicrobial resistance is a worldwide concern that involves not only human and animal bacteria, but also phytopathogenic bacterial strains. It has been reported that the overuse of antibiotics in crops, such as streptomycin, has led to the emergence of resistant phytopathogenic strains, and the severity of the issue has increased. According to the data obtained in the present study, phytopathogenic bacteria showed resistance to antibacterial agents that are not used in agricultural crops [10].

This problem may arise in association with the use of wastewater. Some studies reported that Mexico is among the countries that irrigate agricultural lands with untreated residual water (approximately 387,600 h per year). Some research reported the presence of pollutant organic materials, metals, detergents, antibiotics, and bacteria in residual water, which can cause public health problems and soil degradation, and promote antibiotic resistance in crops [57].

In some studies around the world, it was reported that wastewater treatment plants release antibiotics into the environment. In this sense, Bougnom et al. (2019) suggested that wastewater is associated with the generation and dissemination of antibiotic resistance, since drug-resistant plasmids and antibiotic resistance genes have been identified in wastewater. These genes may encode resistance to trimethoprim, aminoglycosides, β-lactams, macrolides, quinolones, amphenicols, and sulphonamides, which could explain the profiles of resistance to antimicrobials reported in the present study [58–60].

The results of antibiotic sensitivity testing demonstrate the emergence of multi-drug-resistant phytopathogenic strains for which treatment options are limited. In this sense, the hydroalcoholic extract of *L. tridentata* and its organic fraction showed important antibacterial activity.

The antibacterial activity of *L. tridentata* was previously reported Mendez et al. (2012) evaluated four *L. tridentata* leaf extracts (water, ethanol, cocoa butter, and lanolin), and their results revealed that ethanolic extract showed the same activity against Gram-positive and Gram-negative bacteria, similar to the activity observed in our study, with equal MIC and MBC determined for *C. michiganensis* subsp *michiganensis* and *P. syringae* [61].

In another study, Martins et al. (2013) evaluated the antibacterial activity of crude methanolic extract against both Gram-positive and Gram-negative bacteria. They determined a concentration range (MIC) from 62.5 to 250 µg/mL, lower concentrations than those reported in our study (0.39–6.25 mg/mL). Similarly, Snowden et al. (2014) evaluated *L. tridentata* extract (leaves and flowers) against *S. aureus* and obtained MIC of 60 µg/mL [42,43].

Gerstel et al. (2018) tested antibacterial activity against nonantibiotic-resistant and antibiotic-resistant Gram-positive strains, determining an MIC range from 0.35 to 15 µg/mL for *L. tridentata* extract; even though these results cannot be compared with ours, the activity of the species against drug-resistant bacterial strains supports our results [47].

The data obtained in all of these studies support our results, despite the determination of different concentrations. In this sense, it has been reported that solvents used during the extraction process have an influence on the nature and amount of secondary metabolites extracted from medicinal plants. In addition, the yield of extraction depends not only on the solvent, but on the pH, temperature, extraction time, and composition of the sample, and this could explain the different concentrations determined between the studies discussed and the present research [62,63].

L. tridentata aqueous fraction only showed inhibitory activity against *C. michiganensis* subsp *michiganensis* and *P. syringae* (6.25 mg/mL). Organic fraction (ethyl acetate) showed better activity than LTHE and LTAq-F, with an MIC of 3.12 mg/mL against both *C. michiganensis* subsp *michiganensis* and *P. syringae*, and a concentration of 0.39 mg/mL for *X. campestris*.

Martins et al. (2013) evaluated the antibacterial activity of *L. tridentata* methanolic extract and ethyl acetate fraction and determined lower concentrations for the organic fraction. Other studies also determined lower concentrations when ethyl acetate fractions were evaluated, such as those performed by Olmedo-Juarez et al. (2019) and González-Alamilla et al. (2019), which showed lower concentrations of *Caesalpinia coriaria* and *Salix babylonica* ethyl acetate fractions against a wide range of Gram-positive and Gram-negative bacterial strains than those obtained with hydroalcoholic extracts [42,51,52].

Cartaya and Reynaldo (2001) and Martínez-Aguilar et al. (2012) explained that, due to the polarity of ethyl acetate, better extraction could be performed, with higher concentrations of bioactive compounds. Similarly, Gomez-Guiñán et al. (2003) reported that medium and low polarity compounds eluted using ethyl acetate could pass easily through bacterial cell walls, which could explain why better activity was observed with the *L. tridentata* organic fraction [64–66].

With respect to the minimal bactericidal concentrations, obtained in the present study, it was observed that LTHE showed bactericidal activity, with a concentration of 12.5 mg/mL, against *C. michiganensis* sbsp. *michiganensis* and *P. syringae*, and a concentration of 0.78 mg/mL for *X. campestris*. The aqueous fraction did not show any bactericidal effects, while the organic fraction exhibited bactericidal effects on all the evaluated strains, showing MBC values ranging from 0.78 to 6.25 mg/mL.

In the studies carried out by Snowden et al. (2014) and Gerstel et al. (2018), bactericidal effects of *L. tridentata* were observed, but they did not report at which concentrations the activity was determined. The current lack of studies in which the MBC values of plant extracts or fractions are determined limit the discussion of our results [43,47].

The use of bactericidal agents is desirable and preferred, since killing the bacteria allows the infection to be eliminated, and most importantly, minimizes the possible emergence of resistance and the spread of infections; in this sense, *L. tridentata* represents a promising alternative treatment for phytopathogenic bacteria [8,67].

As can be seen, several studies evaluated the antibacterial activity of *L. tridentata*; in some of them, the secondary metabolites associated with its antibacterial activity are reported. The chemical composition of *L. tridentata* indicates the presence of terpenes, saponins, tannins, quercetin, kaempferol, ellagic acid, gallic acid, methyl gallate, resorcinol, cinnamic acid, catechins, and lignans [19,42,45,61,68–71].

Determining the tannin content revealed that *L. tridentata* contains 17.3769 and 37.153 mg/g of hydrolyzable and condensed tannins, respectively; also quantified was a thymol concentration of 4.3033 and a carvacrol concentration of 7.7986 mg/mL. In addition, the total phenolic compounds were estimated, determining an amount of 237.60 mg/GAE/g dry wt. plant in ethanolic extract (70%). Other studies determined the content of quercetin, kaempferol, and Nordihydroguaiaretic acid (NDGA) in methanolic extracts and ethyl acetate fractions, determining concentrations of 8.67, 21.52, and 35.72, and 8.45, 11.89 and 16.51 mg of plant material, respectively [42,45,61,69].

Other studies stated that major antibacterial activity of *L. tridentata* is associated with the presence of lignans, and some of them determined the mechanism of action. Favela-Hernández et al. (2012) reported that the antibacterial activity of chloroform extract from *L. tridentata* leaves was conferred by a lignan identified as 3-demethoxy-6-O-demethylisoguaiacin. The authors reported that the compound is able to affect proteins of the ATP binding cassette transport system, causing bacterial death. Clemente-Soto et al. (2014) determined that MDGA caused membrane destabilization and consequently death of *Mycobacterium tuberculosis*, and similar activity was reported by Guzmán-Beltrán et al.

(2016); these authors found that NDGA in high concentrations inhibited the growth of *M. tuberculosis* [40,41,48,49].

Similarly, Cunningham-Oakes et al. (2015) [44] evaluated the antibacterial activity of NDGA in combination with a commercial antibiotic against *Staphylococcus aureus*, obtaining promising results; this lignan enhanced the antibacterial activity of the commercial antibiotic due to its capacity to damage the bacterial cell membrane. Gnabre et al. (2015) [72] stated that the use of *L. tridentata* lignans could be a way to treat and prevent new diseases, some of which have no cure to date.

According to these reports, we suggest that the antibacterial activity of hydroalcoholic extract and organic fraction of *L. tridentata* could be associated with the presence of lignans, which may exhibit similar mechanisms of action. However, further studies should be performed to identify the bioactive compounds associated with the antibacterial activity reported in the present study.

5. Conclusions

To date, there are no studies reporting the antibacterial activity of *L. tridentata* against multidrug-resistant phytopathogenic bacteria. The present study shows that LTHE and its organic fraction have bactericidal activity in vitro against *Clavibacter michiganensis* sbsp.. *michiganensis*, *Pseudomonas syringae*, and *Xanthomonas campestris*, bacteria that affect tomato (*Solanum lycopersicum* L.) crops. Better results were obtained against *X. campestris*. Therefore, the hydroalcoholic extract of *Larrea tridentata* and its organic fraction could be used as an alternative treatment against diseases caused by these bacteria in crops. However, it is necessary to carry out tests in situ.

Author Contributions: Conceptualization and methodology, N.R.-P., A.L.M.-U., E.A.-T. and A.Z.-B.; validation and formal analysis, N.R.-P., A.Z.-B., A.L.M.-U. and J.P.-M.; investigation and resources, N.R.-P. and A.Z.-B.; data curation, A.Z.-B., A.L.M.-U. and F.A.-R.; writing—original draft preparation, A.L.M.-U., H.F.H. and N.R.-P.; writing—review and editing, A.Z.-B., F.A.-R., E.A-T. and G.E.-S.B.; supervision, N.R.-P. and A.Z.-B.; project administration, N.R.-P. and A.Z.-B.; funding acquisition, N.R.-P. and A.Z.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to acknowledge the Universidad Autónoma del Estado de Hidalgo (UAEH) for the support provided to carry out the study at its facilities. This study forms part of the thesis of Ana Lizet Ubaldo Morales (Maestría en Ciencias y Tecnología Agrícola y Forestal Sustentable, Universidad Autónoma del Estado de Hidalgo).

Conflicts of Interest: The authors declare no conflict of interest.

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