

Advances in biology through agronomy, aquaculture, coastal and environmental sciences

Leandris Argentel Martínez
Ofelda Peñuelas Rubio

Editors



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**Leandris Argentel Martínez
Ofelda Peñuelas Rubio**

**Advances in biology through
agronomy, aquaculture, coastal and
environmental sciences**



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Prologue

Advances in biology through agronomy, aquaculture, coastal and environmental sciences is an electronic book, edited by Pantanal Editora, based on the compilation of research papers where the authors of the different chapters have used highly current scientific methodologies and research equipment.

The biological sciences as the main object of research in agriculture, aquaculture, coastal and environmental sciences generate every day an understanding of knowledge that allows raising the scientific level of society as part of universal access to knowledge.

This book mainly addresses issues related to the use of plants extracts as sustainable alternatives for biocontrol of pests and bacterial diseases. It also brings together information on viruses and other diseases in aquatic organisms. In addition, studies of mangroves structure and their contribution to carbon sinks in experimental sites in northwestern Mexico are presented. Finally, an analysis on educational strategies for environmental education based on plant biology is carried out.

Editors appreciate the participation of the authors who have come from higher education institutions and research centers of great scientific prestige in Mexico. The majority of them are members of the National Research System of CONACyT, Mexico.

The authors


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Evaluation of the antioxidant and antimicrobial activity of hydroalcoholic extracts of *Larrea tridentata* leaves

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ABSTRACT

Approximately 80% of the world's traditional medicines use plant species to meet primary health care needs. In recent years, it has been discovered that the essential oil compounds of *larrea tridentata* have beneficial activities: antioxidant, antitumor, neuroprotective, regenerative, antibacterial, antiviral, antifungal, anthelmintic, antiprotozoal and insecticidal activities. However, few investigations have evaluated the secondary metabolites of their ethanolic extracts. The objective of present study is evaluation of the antioxidant and antimicrobial activity of hydroalcoholic extracts of *larrea tridentata* leaves. The ethanolic extracts evaluated contained 25.08 ± 2.47 mg of anthocyanins 100 g^{-1} of plant and 228.88 ± 3.45 mg gae polyphenols 100 g^{-1} of plant. The antioxidant activity evaluated by dpph is $47.12 \pm 2.43\%$. The ethanolic extracts had a high efficiency in inhibiting the growth of *staphylococcus aureus*, *escherichia coli*, *salmonella* and *shigella*, with inhibition halos of 13.41 ± 0.51 to 19.67 ± 0.41 mm. The determination of the minimum inhibitory concentration (mic) was $0.5 \mu\text{g ml}^{-1}$ against *escherichia coli*, $7.74 \mu\text{g ml}^{-1}$ against *salmonella*, $9.14 \mu\text{g ml}^{-1}$ against *shigella* and $12.25 \mu\text{g ml}^{-1}$ against *staphylococcus aureus*. These results showed that the tested ethanolic extracts possess antibacterial activities against bacteria that cause foodborne illness.

Keywords: foodborne illness; inhibition halos; anthocyanins; polyphenols; gae polyphenols; minimum inhibitory concentration (mic).

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INTRODUCTION

Human beings have used medicinal plants since ancient times to meet primary health care needs. In general, these plants are one of the main sources of phytochemicals (Martins et al., 2013). Approximately 80% of the world's traditional medicines are made up of bioactive compounds found in plants (Oliveira et al., 2005). Due to the biodiversity present on our planet, science has shown that many plants can be used in medicine (Corell-Doménech, 2019). *L. tridentata*, commonly known as gobernadora, is a plant that grows in the northern part of Mexico and the southwestern United States (Ross, 2005).

Mexico and especially the state of Sonora have great biodiversity, with more than 26,500 species of flowering plants, 1,600 ferns, 976 species of mosses and 1,700 species of lichens recorded. Several cultures have used diverse plant species, including *L. tridentata*, for medicinal purposes (Andrango-Quisaguano, 2022). Today, scientific reports on these species are very limited, with less than 10% of the world's angiosperm species evaluated for their chemical composition and pharmacological properties, which motivates research on medicinal plants, as they possess valuable phytochemical and pharmacological information, becoming important in modern medicine. Other species can be a direct source of therapeutic agents as raw material for the manufacture of semisynthetic drugs. The chemical structure of their active principles can serve as a model for the development of synthetic drugs, and these principles can be used as taxonomic markers in the search for new drugs (Marcano; Hasegawa, 2018).

The increase in diseases caused by pathogenic microorganisms is a generalized concern and constitutes a risk factor for public health, which is why compounds from natural sources that inhibit bacterial growth are being sought (Corzo-Barragán, 2012). *L. tridentata* is an exceptional source of polyphenols, and its main exponent in this plant is nordihydroguaiaretic lignan (NDGA), which has been noted as being responsible for the biological activity of *L. tridentata* (Lambert et al., 2004; Martins et al., 2013).

However, the secondary metabolites found in *L. tridentata* leaves include lignans, flavonoids, saponins, triterpenes and triterpenoids, among others. Most of these compounds are studied for their antimicrobial, antiviral (Reyes-Melo et al., 2021), antifungal (Tequida-Meneses et al., 2002; Tucuch-Pérez et al., 2020) and antibacterial (Núñez-Mojica et al., 2021) properties. Nevertheless, only a few studies have reported the antibacterial and antioxidant activity of ethanolic extracts of *L. tridentata* leaves. These types of plants are an important source of antioxidants, which have an impact on food quality and consumer health.

MATERIALS AND METHODS

Experimental area

The equivalent of 2 kg of governor's weed was used. The grass was obtained in the proximity of Ciudad Obregón, Sonora. With coordinates 27°28'55.9 "N 110°00'02.0 "W. Once the plant was collected, it was transported to the Tecnológico Nacional de México campus Valle del Yaqui, located at Avenida

Tecnológico, Block 611, Bácum, Sonora, México. C.P. 85276, to the ultrasonic pulse laboratory and the aquaculture laboratory. These laboratories have the appropriate equipment for this research.

Extraction of phytochemicals from *L. tridentata*

For the extraction of phytochemicals, an ethanol-acetic acid-water solution was prepared in a 50:10:40 v/v ratio for both corns in a 1:30 w/v ratio, and the pH was adjusted to 1 by adding 2 molar acetic acid and placed in constant agitation for 15 days. Finally, the sample was centrifuged at 4000 g, and the supernatant was collected. The extract obtained was concentrated to dryness in a Rotavapor (DLAB model RE100-S) at 50 °C and stored in the dark at -20 °C until use. The total phenol content was expressed as mg 100 g⁻¹ (Abdel-Aal; Hucl, 1999; Ju; Howard, 2003).

Determination of total phenols

Based on the determination of Singleton and Rossi (1965). For the reaction, 90 µL of a 50 mg mL⁻¹ solution of extract (per genotype) was added, 1.91 mL of deionized water and 200 µL of Folin-Ciocalteu's reagent were mixed; after 2 minutes, 0.8 mL of 15.9% sodium carbonate was added and then incubated at a temperature of 50 °C for five minutes, and the absorbance was measured at 765 nm. The total phenol content was expressed as mg gallic acid equivalent (GAE). 100 g⁻¹ dry extract (Pérez-Pérez et al., 2014; Singleton; Rossi, 1965).

Determination of total flavonoids

For this variable, 0.5 mg mL⁻¹ of the extract in solution was mixed with 2 mL of distilled water and 150 µL of sodium nitrite, left to stand for five minutes by adding 150 µL of a 2.5% aluminum chloride solution, and then left to stand for six minutes by adding 1 mL of 0.5 N sodium hydroxide and 5 mL of distilled water, the absorbance was measured at 510 nm. To determine the flavonoid content, a calibration curve with quercetin was generated, and the total flavonoid content was expressed as mg of kersetin 100 g⁻¹ of dry extract (Reyes et al., 2013; Zhishen et al., 1999).

Antioxidative capacity by DPPH

The capacity to scavenge the DPPH radical (2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazin-1-il) is based on the reduction of the absorbance at 517 nm by the action of antioxidants. The performance of this experiment consisted of mixing 3.9 mL of 100 µM DPPH radical dissolved in 80% methanol with 0.1 mL of the sample or standard and kept in the dark for 30 minutes. The reading was made at 517 nm after an incubation period of 30 minutes, and the results were expressed in % inhibition (Molyneux, 2004).

Antimicrobial activity by disk diffusion

The qualitative analysis of the antimicrobial activity was performed using the disk diffusion method, and the bacteria used were the following: *Staphylococcus aureus* (ATCC 8532), *Escherichia coli* (ATCC 12210), *Salmonella* (ATCC 8230) and *Shigella* (ATCC 4837). Bacteria were grown in 2% (w/v) trypticase soy broth (TSB). Purity control assays were used to verify the status of the bacteria. The strains were preserved in TSB broth with 20% sterile glycerol (v/v) at -20 °C (Ma et al., 2019).

The procedure was performed by impregnating sterile 6 mm diameter Whatman No. 1 filter paper discs with a concentration of 10 mg/disc of extract. Gentamicin was used as a positive control (30 µg/disc), and sterile distilled water was used as a negative control. The inhibition halo was measured after 24 hours of incubation at 37 °C. The criterion for interpretation was gentamicin (450 µg disc⁻¹) as a reference. Subsequently, inhibition halos were measured starting from the center of the discs to the end of the inhibition halo (Riverón-Rodríguez et al., 2012).

Statistical analysis

Biochemical analyses were performed in triplicate using Statgraphics Software (Version 18) to calculate the mean and standard deviation. The significant difference between two mean values was determined by analysis of variance (ANOVA) at the 95% confidence level ($p \leq 0.05$), and the significant difference between groups was determined by Tukey's comparison of means ($p < 0.05$).

RESULTS AND DISCUSSION

Table 1 shows the concentrations of polyphenols extracted from *L. tridentata*, evidencing the presence of 228.88 ± 3.45 mg of GAE 100 g⁻¹ of plant. One possible justification for this high value is the environmental and surface conditions. This is because the environment directly affects a plant's ability to produce phytochemicals (Björkman et al., 2011). Studies on medicinal plants have found that factors such as water stress and temperature influence the phytochemical composition of medicinal plants (Shin et al., 2021). They also found that secondary metabolites play an important role in plant adaptation to the environment and plant recovery under stress conditions (Oh et al., 2009). Among the different types of stresses, water shortage, temperature and salt water are considered negative factors, as they lead to reduced yields of various crops. However, plants grown under dry conditions often produce higher concentrations of active ingredients that protect against free radicals and reactive oxygen species and help prevent photosynthetic damage (Albergaria et al., 2020; Oh et al., 2009).

The total amount of anthocyanins obtained from ethanol was higher than that reported for *L. tridentata* elsewhere in Mexico (Saldívar-Lira, 2003). With respect to food technology and the use of medicinal herbs, it has been found that adding herbs to juice reduces the anthocyanin content due to the soaking effect of the extract on the phytochemicals in dried herbs (Teneva et al., 2022). Therefore, it is difficult to retain sufficient anthocyanins in the juice. However, although it is difficult to link the increase

in anthocyanins with their stability, it is certain that plant extracts can protect juice components from decomposition during processing. However, the effectiveness of phenolic compounds in food preparations was not related to the polyphenol content or the resulting antioxidant properties but to their quality, suggesting that the phenolic composition is qualitative. was more important than the total polyphenol content of the extract (Klisurova et al., 2019).

Table 1. Anthocyanin composition, polyphenols and antioxidant activity of gobernadora.

	Anthocyanins mg 100 g⁻¹ de plant	Polyphenols mg of GAE 100 g⁻¹ de plant	DPPH (% of inhibition)
<i>L. tridentata</i>	25.08±2.47	228.88±3.45	47.12±2.43

¹ Gallic Acid Equivalents (GAE); 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The total amount of anthocyanins obtained from the extract was superior to that reported for *L. tridentata* in other places of Mexico (Saldívar-Lira, 2003; Urias-Lugo et al., 2015). With respect to food technology and the use of medicinal herbs, it has been found that adding herbs to juice reduces the anthocyanin content due to the soaking effect of the extract on the phytochemicals in dried herbs (Teneva et al., 2022). Therefore, it is difficult to retain sufficient anthocyanins in the juice. Nevertheless, the effectiveness of phenolic compounds in food preparations was not related to the polyphenol content or the resulting antioxidant properties but to their quality, suggesting that the phenolic composition is qualitative. was more important than the total polyphenol content of the extract (Klisurova et al., 2019).

Antimicrobial activity

The bioassays were evaluated with strains of *Staphylococcus aureus* (ATCC 8532), *Escherichia coli* (ATCC 12210), *Salmonella* (ATCC 8230) and *Shigella* (ATCC 4837). The inhibition range shown is between 13.41 and 19.67 mm. This was compared to the gentamicin positive control, where an inhibitory halo of 16.01±1.17 to 22.47±0.41 mm was observed, and no inhibition was observed in the water negative control. However, it is important to note that the dose of gentamicin is 22.5 times the amount of plant extract used. A recent study demonstrated the in vitro growth inhibitory effect of 14 strains of *Xanthomonas axonopodis* using methanolic extracts of leaves and stems of *Bixa orellana*, *Gliricidia sepium*, *Ocimum basilicum* and *Petiveria alliacea*. The study concluded that these four plants have fungicidal effects and may be promising for controlling plant diseases (Nalimova et al., 2005). The inhibition halos reported were 17 to 21 mm in diameter for the four extracts used, very similar to the results obtained in this study (Table 2). Martínez-Valverde and Colmenares (1999) studied the effects of several extracts, including eucalyptus and *L. tridentata*, and found that the growth of pathogen colonies was inhibited by 90-100%. Similarly, results were obtained for 7 monocots, 46 dicots, 1 gymnosperm and 2 ferns. However, they reported that only nine showed in vitro growth inhibition of common pathogens. These inhibitory halos

were similar to eucalyptus extract (12.4 mm) and palo santo extract (11.3 mm) (Martínez-Valverde et al., 2000; Stauffer et al., 2000).

Table 2. Inhibition of extracts against pathogens.

	Inhibition zone size (mm)			
	Bacteria			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella</i>	<i>Shigella</i>
<i>L. tridentata</i> extract	12.25 ^b	0.5 ^c	7.74 ^a	9.14 ^a
Gentamicin	21.35 ^a	19.88 ^b	14.21 ^c	17.44 ^b
Water	NA	NA	NA	NA

¹NA, no activity; Values are expressed in $\mu\text{g mL}^{-1}$. Different letters in the columns indicate significant differences ($p \leq 0.05$)

The antimicrobial efficacy of *L. tridentata* extracts is shown in Table 3. The lowest concentration was observed for *Escherichia coli* at 0.5 mg mL^{-1} , which agrees with the sensitivity and inhibition rate reported in Table 2. The other bacteria were affected by the extract of *L. tridentata*. However, the highest minimum concentration was for *Staphylococcus aureus* (ATCC 8532). Previous studies found that the antibacterial activity of the plant was equal for gram-positive and gram-negative bacteria (Mendez et al., 2012). The range of action of antibacterial activity in crude methanol extracts ranges from 62.5 to $250 \mu\text{g mL}^{-1}$ (Snowden et al., 2014). However, other authors have found inhibition ranges from 0.35 to $15 \mu\text{g mL}^{-1}$ (Gerstel et al., 2018), similar to the concentrations reported in our study (0.5 - 12.5 g mL^{-1}). In addition, the evaluation of *L. tridentata* flower extracts against *Staphylococcus aureus* yielded an MIC of $60 \mu\text{g mL}^{-1}$ (Gerstel et al., 2018).

Although the concentrations between studies differ from one another, the antibacterial activity is still present. Different factors can influence the nature and quantity of the secondary metabolites extracted, among the most important of which are solvent, pH, temperature, extraction time and sample composition, which could explain the different concentrations determined among the different studies, including the present investigation (Dirar et al., 2019; Do et al., 2014). Among the wide variety of bacteria to which *L. tridentata* has shown inhibitory activity are *Clavibacter michiganensis* subspecies *michiganensis* and *P. syringae* at a concentration of 6.25 mg mL^{-1} . However, the concentration reported for these species is higher than the range of action of the antibacterial activity. This difference may be related to the extraction solvent (Morales-Ubaldo et al., 2021). The low concentrations to inhibit bacteria may be associated with the polarity of the compounds extracted with different solvents; for example, compounds extracted with ethyl acetate can easily penetrate bacterial cell walls and, therefore, can disrupt the barrier function of the cell membrane or cause membrane fusion, a process that results in the leakage of intramembranous materials. This mechanism of action decreases the necessary concentration of the compound to inhibit a specific bacterium (Renzetti et al., 2020); therefore, compounds of medium and

low polarity may have a higher activity against microorganisms (Gómez-Guiñán et al., 2003; Lobiuc et al., 2023; Martínez-Aguilar et al., 2012).

Table 3. Inhibitory minimum concentration.

	Concentration ($\mu\text{g mL}^{-1}$)	Inhibition zone size (mm)			
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella</i>	<i>Shigella</i>
<i>L. tridentata</i> extract	20 \pm 0.45	13.41 \pm 0.51 ^a	17.33 \pm 1.54 ^a	14.67 \pm 0.90 ^a	19.67 \pm 0.41 ^a
Gentamicin	450 \pm 1.53	21.11 \pm 1.21 ^b	21.04 \pm 0.58 ^b	16.01 \pm 1.17 ^a	22.47 \pm 0.41 ^b
Water	0	0	0	0	0

¹Different letters in the columns indicate significant differences ($p \leq 0.05$)

The use of natural extracts is a promising alternative in the control of bacterial infections (Esposito; Turku, 2023). There is considerable variation among the results in the study of secondary metabolites of *L. tridentata* because of the variety of phytochemicals contained in the plant (Qaderi et al., 2023; Reyes-Melo et al., 2021). Some secondary metabolites reported are terpenes, saponins, tannins, quercetin, kaempferol, ellagic acid, gallic acid, methyl gallate, resorcinol, cinnamic acid, catechins and lignans. However, these vary according to the environmental conditions of the area. In conclusion, phytochemicals exist in nature as nutraceuticals that are beneficial to human health, especially for their antioxidant and antimicrobial effects. The great variety of different compounds present in plants provide a rich source of potential pharmaceuticals to improve human health. Moreover, these compounds are very diverse in their mode of action, making them unlikely to cause bacterial resistance.

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