



## Antimicrobial activity and ricinine quantification in *Ricinus communis* against phytopathogenic bacteria

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### ABSTRACT

**Background/Objective.** Metabolites of castor bean (*Ricinus communis*), such as the alkaloid ricinine, have antibacterial properties with the potential to combat phytopathogenic bacteria such as *Xanthomonas perforans*, *Acidovorax citrulli*, *Clavibacter michiganensis*, and *Ralstonia solanacearum*, making it a natural alternative to conventional chemicals. Therefore, the objective of this study was to evaluate the antimicrobial activity of methanolic extracts from castor bean leaf accessions and quantify ricinine using HPTLC to determine its effectiveness as a bactericidal agent.

**Experimental development.** The leaves of nine castor bean accessions were ground and sieved to prepare methanolic extracts coded as: 1+, 2+, 4+, 5+, 6+, 7+, 8+, 9+, and 10+. Ricinine in the extracts was quantified by HPTLC. To determine antibacterial activity, the phytopathogenic bacteria *Xanthomonas perforans*, *Acidovorax citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, and *Ralstonia solanacearum* were reactivated and cultured in selective medium with concentrations of the extracts at 100, 75, 50, and 25% and a chemical control Finalbacter® (Gentamicin Sulfate and Oxytetracycline Hydrochloride). The analysis was performed in triplicate.

**Results.** The extracts identified as 2+ and 5+ proved to be the most effective, with inhibition halos of 18.94 mm and 17.23 mm, respectively. *Ralstonia solanacearum* showed the greatest inhibition (21.6 mm), followed by *A. citrulli* and *X. perforans* (19.26 mm each) and *C. michiganensis* subsp. *michiganensis* (12.21 mm). The 100% and 75% concentrations showed the largest inhibition halos (20.6 mm and 19.8 mm), compared to the lower concentrations and the chemical control. Ricinine concentrations ranging from 0.88 to 2.14 mg g<sup>-1</sup> were quantified.

**Conclusion.** Accessions 2+ and 5+ of *Ricinus communis* showed potential as a natural option for controlling phytopathogenic bacteria, with an average inhibition zone of 19.31 mm and 17.92 mm, respectively.

**Keywords:** Castor bean, Bioactive compounds, Plant pathology, Methanolic extracts



## INTRODUCTION

Castor bean (*Ricinus communis*), also known as castor oil plant, is a perennial shrub belonging to the family Euphorbiaceae, widely distributed in tropical and subtropical regions around the world. This shrub is notable for its adaptability to various climates, including tropical, subtropical, and warm temperate conditions. In Mexico, it occurs in tropical areas of the south and semi-arid regions of the north, both in the wild and under commercial cultivation (Gómez-González *et al.*, 2020).

The castor seed is rich in oil, with a content ranging from 40 to 60%. The demand for castor oil has shown an annual growth of 3 to 5% due to its economic importance, as its lubricating properties make it valuable in the pharmaceutical, cosmetic, and biodiesel industries (Suryaningsih *et al.*, 2025). In addition, it is used in traditional medicine for its laxative, analgesic, anti-inflammatory, and antimicrobial properties (Kaur and Bhaskar, 2020; Cheikhoussef *et al.*, 2023). Several metabolites have been identified in *Ricinus communis*, including the alkaloid ricinine, ricinoleic acid, undecylenic acid, tocopherols, sterols, saponins, and flavonoids (Saravena *et al.*, 2022; Suryaningsih *et al.*, 2025). These bioactive compounds contribute to the antimicrobial activity of *Ricinus communis*; ricinine has been shown to be effective against a variety of pathogens in both plants and humans, suggesting its potential use in the control of bacterial diseases (El-Naggar *et al.*, 2018).

Phytopathogenic bacteria represent a significant threat to agriculture, affecting a wide range of crops and impacting their quality, yield, and overall production. Among the most relevant species are *Xanthomonas perforans*, *Acidovorax citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, and *Ralstonia solanacearum* (Xhemali *et al.*, 2024; Tapia-de la Barrera *et al.*, 2023; Reyes-Tena *et al.*, 2021). *Xanthomonas perforans* is known to cause leaf spot disease in tomato plants, and depending on disease severity and climatic conditions, it can cause yield losses ranging from 23% to 44% (Osdaghi *et al.*, 2021). *A. citrulli* is responsible for bacterial fruit blotch in melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*), which, in cases of infection, can result in total crop loss (Elizalde Jiménez *et al.*, 2011). *Clavibacter michiganensis* subsp. *michiganensis* causes bacterial wilt in tomato crops, with yield reductions of up to 46%, while *R. solanacearum*, the causal agent of bacterial wilt in a wide range of plants, produces economic impacts that depend on the affected crop (Longoria-Espinoza *et al.*, 2020; Soto-Caro *et al.*, 2023).

The use of castor bean extracts to combat these phytopathogenic bacteria offers a promising alternative to the conventional use of pesticides and antibiotics. The identification and characterization of the metabolites responsible for antimicrobial activity, using advanced techniques such as high-performance thin-layer chromatography (HPTLC), can provide valuable information for the development of alternative treatments. Therefore, the objective of this study was to quantify ricinine in nine castor bean accessions using HPTLC and to evaluate their bactericidal potential against the main bacteria associated with phytopathogenic diseases.

## EXPERIMENTAL DEVELOPMENT

**Sample collection and preparation of plant extracts.** Nine naturalized *Ricinus communis* accessions from Mexico were evaluated. Samples were collected in March 2022 from a germplasm field established in Imala, Culiacán, Sinaloa (33° 34' 00.500" S, 70° 38' 00.400" W). The germplasm field was established in October 2021 with one accession for every two rows, spaced 25 cm within the row and 90 cm between rows. Weed control was

performed manually, and only compost and biofertilizers produced with mountain microorganisms were applied.

Leaves were collected from the accessions identified as 1+, 2+, 4+, 5+, 6+, 7+, 8+, 9+, and 10+. The leaves were air-dried, then ground using a leaf mill, with cleaning between accessions to avoid cross-contamination. The resulting powder was sieved through a No. 40 mesh. Ten grams of each ground and sieved sample were weighed and placed separately in a flask containing 100 mL of methanol (HPLC grade). The flasks were then kept under constant agitation on an orbital shaker for 48 h at room temperature. Afterward, the extracts were filtered through organza cloth and Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator and stored in amber vials at 4 °C until use.

**Ricinine Quantification by HPTLC.** Using a semi-automatic applicator (Limonat 5, CAMAG), 10 µL of each extract were applied in 8 mm bands onto 20 × 10 cm aluminum TLC Silica Gel 60 F254 plates (in triplicate). The plates were developed in a chromatographic chamber (CAMAG) pre-saturated for 10 min. The mobile phase consisted of toluene–ethyl acetate (9:1, v/v). Once the solvent front reached 75–80 mm, the plate was dried with cold air for 5 minutes. Plates were visualized using a TLC Scanner 4 (CAMAG) at wavelengths of 254 and 366 nm. Both application and scanning were controlled by visionCATS software, version 2.4 (CAMAG, Switzerland).

A calibration curve was prepared with different concentrations of ricinine (methyl-D3): 2, 0.2, 0.02, 0.002, and 0.0002 mg mL<sup>-1</sup>, dissolved in HPLC-grade methanol. Three applications of each concentration were made on a 20 × 10 cm silica gel F254 plate, using 9 mL of toluene and 1 mL of ethyl acetate as the mobile phase.

**Bacterial Reactivation.** Strains of *Xanthomonas perforans*, *Acidovorax citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, and *Ralstonia solanacearum* were obtained from the strain collection of the Phytopathology Laboratory at the Research Center for Food and Development (CIAD), Culiacán Unit. The provided strains had been isolated from different crops exhibiting distinct symptomatologies. Nutrient agar medium was used for strain reactivation, and cultures were incubated at 27 °C until growth was observed. Subsequently, a subculture from a single colony was performed to ensure the purity of each strain.

**Determination of Bacterial Growth Inhibition.** Extracts 2+ and 5+ were selected from a preliminary screening of the nine plant extracts (data not shown). The extracts were diluted in distilled water to concentrations of 100, 75, 50, and 25%. The bactericide Finalbacter® (Gentamicin Sulfate and Oxytetracycline Hydrochloride) was included as a control to evaluate its inhibitory effect in comparison with the plant extracts.

In the procedure, bacterial cultures of *A. citrulli* were prepared on King's B agar and nutrient agar, *C. michiganensis* subsp. *michiganensis* on Mueller–Hinton agar, *R. solanacearum* on King's B agar and CPG agar, and *X. perforans* on nutrient agar. Bacterial suspensions were then prepared by scraping colonies from the culture medium and adding them to test tubes containing sterile distilled water until turbidity was adjusted to level 3 of the McFarland scale (concentration: 9 × 10<sup>8</sup> CFU). Bacterial strains (100 µL of suspension) were inoculated onto plates containing the respective media, and the samples were evenly distributed using glass beads. Subsequently, 5 µL of each extract dilution were applied at three specific points on the plate, in triplicate. The plates were incubated at 27 °C for 48 hours to assess bacterial growth inhibition.

**Statistical Analysis.** The data obtained were analyzed using one-way ANOVA with a significance level of  $p < 0.05$ , and mean comparisons were performed using Tukey's test with the statistical software MINITAB 17.

**Quantification of Ricinine by HPTLC.** According to the results of the ricinine analysis by HPTLC, no statistically significant difference was observed among the different concentrations ( $p = 0.066$ ). Concentrations ranged from 0.88 (4+) to 2.14 mg g<sup>-1</sup> (1+) (Table 1). These results are consistent with those reported for the *Ricinus communis* variety Guanajuatol (Zavala-Gómez *et al.*, 2021), although they differ in the plant tissue used for extraction; ricinine is present in all parts of the plant (Nour *et al.*, 2023).

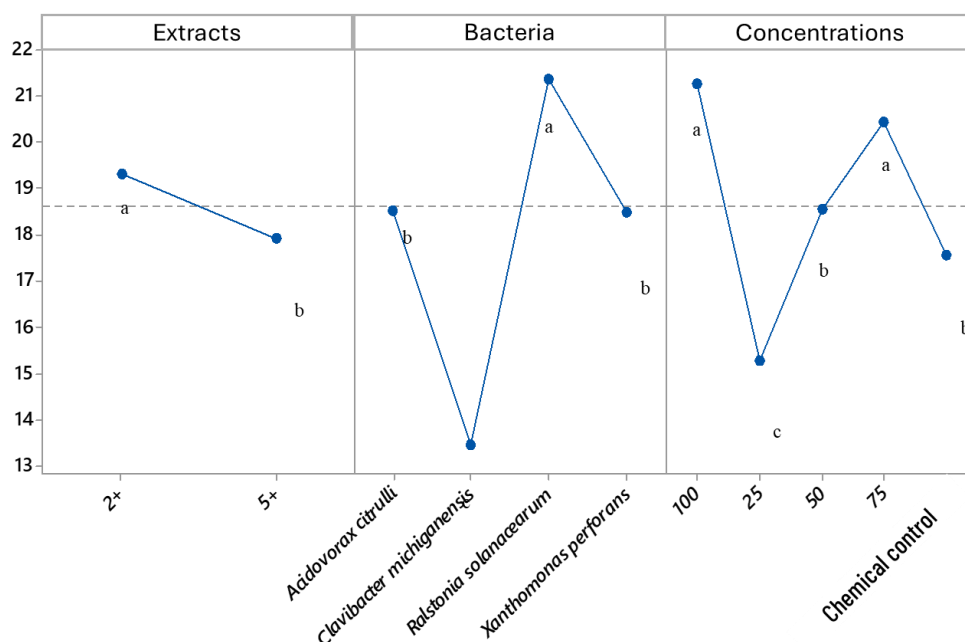
Ricinine quantification is essential for identifying potential varieties with higher concentrations of this bioactive compound and selecting the most effective ones to produce extracts and derived products, thereby optimizing their use in agriculture and medicine (Zheng *et al.*, 2023). This information is also valuable for breeding programs, the adjustment of cultivation practices, and the maximization of commercial value (Acosta-Navarrete *et al.*, 2023; Leal *et al.*, 2022). Moreover, it ensures the safety and efficacy of ricinine applications, since high concentrations can be toxic (Staňková *et al.*, 2020).

**Table 1.** Quantification of ricinine in methanolic leaf extracts from nine *Ricinus communis* accessions.

Accession	Quantification (mg g <sup>-1</sup> ) mean ± SD <sup>z</sup>
1+	2.14±0.75 <sup>a</sup>
2+	1.58±0.15 <sup>a</sup>
4+	0.88±0.09 <sup>a</sup>
5+	1.21±0.49 <sup>a</sup>
6+	1.89±0.95 <sup>a</sup>
7+	1.70±0.21 <sup>a</sup>
8+	1.53±0.07 <sup>a</sup>
9+	1.49±0.01 <sup>a</sup>
10+	1.03±0.20 <sup>a</sup>

<sup>z</sup>SD = Standard deviation. Mean ± SD. Identical letters indicate no significant differences (Tukey,  $p > 0.05$ ).

**Antimicrobial activity of methanolic extracts of *Ricinus communis*.** The statistical analysis revealed significant differences in the evaluated parameters, as shown in the main effects plot (Figure 1).



**Figure 1.** Main effects plot of methanolic leaf extracts of *Ricinus communis*, bacteria (*Xanthomonas perforans*, *Acidovorax citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, and *Ralstonia solanacearum*), and extract concentrations. Different letters among sections indicate statistical differences.

According to the preliminary results, extracts from accessions 2+ and 5+ exhibited the greatest antimicrobial effectiveness. In contrast, extract 1+, despite showing the highest ricinine concentration, did not display significant antimicrobial activity, suggesting that this alkaloid alone is not primarily responsible for the antimicrobial effect. This indicates that other bioactive compounds in the extracts may be involved in the observed antimicrobial activity. Studies by Hussein *et al.* (2018) and Abomughaid *et al.* (2024) have reported that alcoholic leaf extracts of *R. communis* contain phytochemicals such as flavonoids, terpenes, and tannins, which are known for their antimicrobial activity and well-defined mechanisms of action. Therefore, the efficacy of extracts 2+ and 5+ may be attributed to the combined or synergistic presence of these secondary metabolites. Further studies on their compounds are therefore necessary.

A statistically significant difference was found among the extracts evaluated against the bacterial isolates. On average, extract 2+ produced the largest inhibition zone (19.31 mm), followed by extract 5+, which produced an inhibition zone of 17.92 mm. Antibacterial activity was evidenced by the presence of well-defined inhibition halos at the different concentrations tested (Figure 2). Likewise, a significant difference was observed among the bacterial species studied: *R. solanacearum* showed the greatest inhibition (21.36 mm), followed by *A. citrulli* and *X. perforans*, both with 18.5 mm. Finally, *Clavibacter michiganensis* subsp. *michiganensis* exhibited an inhibition of 13.48 mm. These results are consistent with those reported by other authors, who noted that methanolic leaf extracts exhibit the strongest antimicrobial activity, particularly against Gram-negative bacteria (Hussein *et al.*, 2018; Abd-Ulgadir *et al.*, 2015; Al-Kuraishy *et al.*, 2012), as also observed in this study.

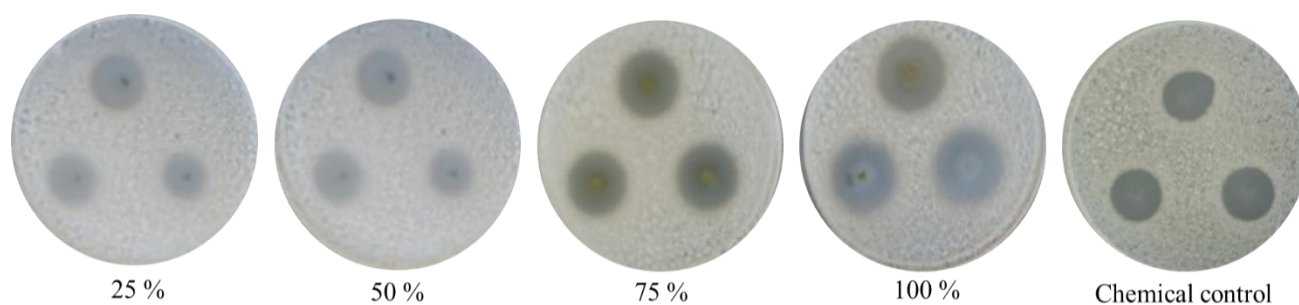
The evaluation of *Ricinus communis* accessions allows for the identification of specific bioactive compounds with antimicrobial potential against phytopathogenic bacteria. In this regard, the results of this study open the possibility of exploring, in future research, their incorporation into new agricultural products such as bioinsecticides, biofungicides, or natural antimicrobials. These products could be formulated together with other proven active ingredients such as garlic (*Allium sativum*), essential oils (Hussein *et al.*, 2025), and even microbial agents such as *Trichoderma* spp. and *Bacillus* spp. (Elhaj *et al.*, 2021), to provide a broader range of solutions for the agricultural market. An example of this is the study conducted by Ramadass and Thiagarajam (2021), who evaluated the antibacterial activity of a *Ricinus communis* formulation for the control of *Xanthomonas oryzae*, demonstrating its bioactive potential. Additionally, *Ricinus communis* extracts have been tested against other phytopathogens such as the nematode *Pratylenchus brachyurus* (Izidoro *et al.*, 2021).

*Ricinus communis* extracts have been evaluated *in vitro* against *Ralstonia solanacearum* isolated from bell pepper, producing inhibition zones of 11.88 mm at 100% concentration (Mayanglambam *et al.*, 2020). Similarly, in this study, a statistically significant difference was found among extract concentrations, with the 100 and 75% concentrations (Table 2) showing the largest inhibition zones, 21.27 and 20.45 mm, respectively. The 50% concentration and the chemical control exhibited inhibition zones of 18.54 and 17.55 mm, respectively, while the 25% concentration showed the smallest inhibition zone (15.27 mm). These results suggest a direct relationship between extract concentration and antimicrobial effectiveness, as lower concentrations and the chemical control produced smaller inhibition zones, underscoring the importance of using adequate concentrations to maximize antimicrobial activity.

**Table 2.** Antimicrobial activity of methanolic extracts from *Ricinus communis* accessions 2+ and 5+ against bacteria associated with plant diseases.

Bacteria	Inhibition zone (mm) (Mean±SD) 2+					Inhibition zone (mm) (Mean±SD) 5+				
	Concentration (%)					Concentration (%)				
	100	75	50	25	CQ	100	75	50	25	CQ
<i>Acidovorax citrulli</i>	21.11a± 2.2	19.96a± 1.9	19.46a ±1.8	14.88 b±1.4	15.47 b±4.0	19.86a b±2	23.06a ±1	18.84a b±1	16.94 b±1.3	14.18b ±4
<i>C. michiganensis</i> subsp. <i>michiganensis</i>	17.24a± 1.05	15.11b± 0.71	13.37c ±1.4	11.18 d±0.5	18.55e ±0.7	11.99 b±1	11.33 b±1	9.21b ±0.8	8.25c ±0.5	18.55a ±0.7
<i>Ralstonia solanacearum</i>	26.59a± 1.17	24.48ab ±0.9	22.78 b±2.4	17.86c ±1.62	18.71c ±4.5	22.31a ±1	20.91a b±1	19.51 b±0.9	16.93c ±1.1	15.72c ±4.2
<i>Xanthomonas perforans</i>	23a±0.5	20.41b± 0.3	20.62 b±0.4	16.17c ±0.8	14.59 d±0.2	22.49a ±1.5	19.93a b±0.5	16.92a b±0.6	14.5b ±1	16.29b ±0.3

\*Different letters within rows indicate statistically significant differences ( $p < 0.05$ ).  $n = 3$ . CQ: chemical control.



**Figure 2.** Antibacterial activity of extract 2+ against *Ralstonia solanacearum*. Clear inhibition zones were observed at different extract concentrations (25, 50, 75, and 100%) compared with the chemical control.

It is important to evaluate *Ricinus communis* leaf extracts for their antimicrobial activity and medicinal properties, as they have been reported to contain bioactive compounds such as ricinine, flavonoids, phenolic acids, and terpenoids (Martínez-Mora *et al.*, 2023), which are effective in treating skin infections such as leishmaniasis and candidiasis (Ghani *et al.*, 2023; Kebede and Shibeshi, 2022; Suurbaar *et al.*, 2017). Although the present study focused exclusively on activity against bacteria associated with plant diseases (*Xanthomonas perforans*, *Acidovorax citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, and *Ralstonia solanacearum*), previous research has shown that these extracts may also exert insecticidal and antifungal effects on agriculturally important pests (Manzoor *et al.*, 2025; Kebede and Shibeshi, 2022; Sotelo-Leyva *et al.*, 2020).

This study represents an important contribution to the field of phytopathology, as it not only confirms the potential of *R. communis* as a source of bioactive antimicrobial compounds but also opens the way for the development of alternative, sustainable agricultural bioinputs that reduce dependence on synthetic agrochemicals. Further research is recommended to deepen the characterization of the metabolites responsible for antimicrobial activity, to study their effects under greenhouse or field conditions, and to extend the evaluation to other pathogens and application methods in order to validate their potential for use in integrated pest and disease management, thereby contributing to sustainability in both the agricultural and health sectors.

## CONCLUSIONS

The methanolic leaf extracts of *Ricinus communis* evaluated in this study demonstrated significant antimicrobial activity against key agricultural bacteria such as *Ralstonia solanacearum*, *Acidovorax citrulli*, and *Xanthomonas perforans*. Accessions 2+ and 5+ showed the greatest effectiveness, with average inhibition zones of 19.31 mm and 17.92 mm, respectively, highlighting their potential as natural bactericidal agents for the integrated management of these phytopathogenic bacteria. Ricinine quantification by HPTLC revealed variations in the concentration of this alkaloid among accessions; however, the lack of a direct correlation with antibacterial activity suggests the likely involvement of other secondary metabolites in bacterial inhibition, offering a novel perspective on the complexity of the bioactive profile of *Ricinus communis*.

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