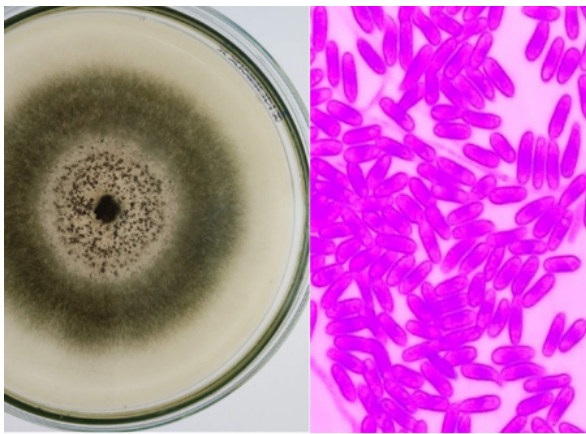


# *In Vitro* antifungal activity of ethanol extracts from *Cnidoscolus urens* L. in controlling *Colletotrichum* spp. in *Lycopersicum esculentum*: a sustainable agricultural perspective

Actividad antifúngica *In Vitro* de extractos etanólicos de *Cnidoscolus urens* L. en el control de *Colletotrichum* spp. en *Lycopersicum esculentum*: una perspectiva agrícola sostenible



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*Colletotrichum* spp. aislado de tomate de mesa.

Foto: M.F. Carrillo

## ABSTRACT

Anthraxnose, caused by the pathogenic fungi *Colletotrichum* spp., poses a significant threat to table tomato (*Lycopersicum esculentum*) cultivation. This study delves into the potential of plant extracts from *Cnidoscolus urens* L. as an alternative biocontrol strategy to combat this disease. Rich in secondary metabolites like terpenes, which are instrumental in plant defense, these extracts also comprise esters and fatty acids. Although the latter are not classified as secondary metabolites, they contribute significantly to the plant's biochemical makeup. Our objective was to gauge the *in vitro* inhibitory efficacy of ethanolic extracts derived from the leaves and stems of *Cnidoscolus urens* L. against *Colletotrichum* spp. To achieve this, an agar dilution method with varying extract concentrations was employed. The results showed that concentrations ranging from 1:0 to 1:5 effectively inhibited fungal mycelial growth. Interestingly, the extracts' origin, whether from leaves or stems, did not show any significant differential impact on their inhibitory activity. These insights emphasize the consistent effect of *Cnidoscolus urens* L. extracts in stalling *Colletotrichum* spp. growth, underscoring their potential as biological antifungal agents in agriculture. Given the pronounced *in vitro* effectiveness of both

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leaf and stem extracts, they beckon further exploration as part of sustainable agricultural strategies to combat prominent diseases like anthracnose.

**Additional key words:** agar dilution method; secondary metabolites; *In Vitro* inhibition; anthracnose biocontrol.

## RESUMEN

La antracnosis, causada por el hongo patógeno *Colletotrichum* spp., representa una amenaza importante para el cultivo del tomate de mesa (*Lycopersicon esculentum*). Este estudio profundiza en el potencial de los extractos vegetales de *Cnidocolus urens* L. como estrategia alternativa de biocontrol para combatir esta enfermedad. Ricos en metabolitos secundarios como los terpenos, que desempeñan un papel decisivo en la defensa de las plantas, estos extractos también contienen ésteres y ácidos grasos. Aunque estos últimos no están clasificados como metabolitos secundarios, contribuyen significativamente a la composición bioquímica de la planta. Nuestro objetivo fue evaluar la eficacia inhibidora *in vitro* de extractos etanólicos derivados de hojas y tallos de *Cnidocolus urens* L. contra *Colletotrichum* spp. Para lograr esto, se empleó un método de dilución en agar con diferentes concentraciones de extracto. Los resultados mostraron que las concentraciones que oscilaban entre 1:0 a 1:5 inhibieron eficazmente el crecimiento del micelio fúngico. Curiosamente, el origen de los extractos, ya sea de hojas o tallos, no mostró ningún impacto diferencial significativo en su actividad inhibidora. Estos conocimientos enfatizan el efecto constante de los extractos de *Cnidocolus urens* L. en el estancamiento de *Colletotrichum* spp. crecimiento, subrayando su potencial como agentes antifúngicos biológicos en la agricultura. Dada la pronunciada eficacia *in vitro* de los extractos de hojas y tallos, invitan a una mayor exploración como parte de estrategias agrícolas sostenibles para combatir enfermedades importantes como la antracnosis.

**Palabras clave adicionales:** método de dilución en agar; metabolitos secundarios; inhibición *In Vitro*; biocontrol de la antracnosis.

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## INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is a perennial plant often cultivated as an annual, with a bush-like growth pattern. It presents high genetic diversity, with countless varieties differing in appearance, color, and taste. This crop continues to experience increasing demand worldwide, underlining its importance in production and commercialization (Waheed *et al.*, 2020). Tomatoes thrive in warm to moderate climates but are susceptible to frost and low temperatures. They can be cultivated in greenhouses or outdoors, needing good soil drainage and high organic matter content. However, if exposed to excessively low or high temperatures, the plant progressively weakens, potentially leading to death (Alarcon *et al.*, 2020).

In Colombia, tomato production is commonplace across the country but is primarily concentrated in Antioquia, Norte de Santander, Valle de Cauca, Boyaca, Huila, Cundinamarca, Risaralda, and Caldas departments, spanning approximately 15,849 ha (Sepúlveda-Flórez, 2016). However, tomato cultivation faces significant challenges from various pests and diseases, including physiological disorders not caused by pathogens or insects, resulting in an increasing reliance on agrochemicals and affected yields (Panno *et al.*, 2021). One such notable disease is anthracnose, a fungal disease causing substantial yield loss and significant damage to fruits (Shahriar *et al.*, 2023). The disease symptoms can be present on

fruits, stems, and branches at different phenological stages, particularly during rainy periods with average temperatures between 13 to 15°C and 95% environmental humidity.

Anthraxnose in Colombia has been reported to be caused by the *Colletotrichum acutatum*, *C. boninense*, and *C. gloeosporioides*, based on their morphological characterization. Although traditional methods to mitigate, control, and eradicate *Colletotrichum* spp. populations are employed, and the use of chemical compounds such as Benomil50® (AgroIQC) poses environmental and health issues and affects microbial diversity. In recent years, bioactive organic compounds extracted from plants have been used to control pathogenic microorganisms due to their antifungal properties, thus reducing the environmental impact of fumigation (Kong *et al.*, 2020). Anthracnose, causes typical necrotic lesions on the stems, leaves, and fruits, has a considerable relevance in agriculture. It poses the potential to destroy up to 100% of the harvest and frequently results in chronic yield reduction (Ciofini *et al.*, 2022). This phytopathology, caused by *Colletotrichum* spp., is one of the main constraints in tomato cultivation and often occurs during rainy seasons and under high relative humidity conditions, damaging leaves and fruits at any phenological stage, and also in postharvest (Ciofini *et al.*, 2022).

Fungal control is typically carried out with chemical products that can be highly toxic and contribute to environmental deterioration and subsequently poor fruit quality. Owing to the buildup of disease resistance from the overuse of synthetic fungicides, there is a growing recommendation for alternative disease management strategies (Percival and Graham, 2021). Recent research focuses on evaluating alternative controls, such as antagonist microorganisms, physical treatments, and natural substances like essential oils and different types of extracts to reduce dependence on these fungicides (El Khetabi *et al.*, 2022). Phytopathogen control with plant extracts is mainly due to the presence of secondary metabolites synthesized in plants as part of their defense mechanism. The diversity of components in the extracts explains their broad spectrum of biological activity (Elhamuly *et al.*, 2022).

Plant extracts from several *Cnidoscopus* species have been reported to disrupt the biological cycles of pests

and diseases caused by fungi and bacteria in various crops. *Cnidoscopus urens* L., a species with significant nutritional and medicinal potential, belongs to the Euphorbiaceae family and exhibits a biochemical composition based on anthocyanins, anthraquinones, coumarins, flavonoids, steroids, lignans, saponins, tannins, terpenoids, xanthines, and alkaloids (Carvalho Neto *et al.*, 2018). This plant has attracted the attention of the scientific world, primarily due to the multiple biological actions it can perform. Research involving plant extracts as medicine is of increasing interest due to the progressive information on the antimicrobial activity of crude extracts (Chaves-Bedoya, 2022).

In the contemporary agricultural landscape, the cultivation of table tomatoes (*Lycopersicon esculentum*) faces significant threats from the phytopathogenic fungus *Colletotrichum* spp., which is responsible for anthracnose, a disease causing substantial economic losses regionally. Given this scenario, the search for new, sustainable, and environmentally benign alternatives to control this pathogen becomes imperative. In this context, our research spotlighted plant extracts from the medicinal plant *C. urens* as a promising contender, building on its reported potent biological activity against a variety of microorganisms.

The aim of this study was to evaluate the *in vitro* effect of ethanol extracts of *C. urens* L. on *Colletotrichum* spp. primarily responsible for postharvest anthracnose disease in tomato crops. This study favors sustainable development and the use of the region's natural resources, offering alternatives to be incorporated into agroecological management to reduce the use of chemical fungicides in combating diseases in agricultural production.

## MATERIALS AND METHODS

### Collection of *Cnidoscopus urens* L.

Vegetal material (leaves and stems) of *Cnidoscopus urens* L. was collected in the municipality of Cucuta (Norte de Santander-Colombia), at coordinates 7°52'56.4" N 72°33'06.2" W. Each sample was transported to the research laboratory Fitobiomol and stored at -80°C until the moment of processing the samples (Fig. 1).



**Figure 1. Collection and preparation process for the ethanol extract of *C. urens* (commonly known as "pringamosa"). (A) Milky latex of *C. urens* on the stem, with visible trichomes. (B) Samples stored in paper bags; note that collectors use gloves to prevent irritation from the stinging trichomes. (C) Leaves and flower of *C. urens*. Photos: Giovanni Chaves-Bedoya**

### Preparation of ethanolic extracts

Dry vegetative material from *C. urens* was used, stored in envelopes containing 100 g of each sample and kept in a freezer at  $-20^{\circ}\text{C}$  for subsequent lyophilization. This process involves removing water from the vegetative material through freezing. After 24 h of lyophilization, each sample of the dried, crushed vegetative material was ground and weighed. A 96% ethanol solution (Merck, Germany) was added at a 1:6 weight/volume. The samples were then agitated for 24 h in total darkness on a shaker (MAXQ 4450, Thermo Scientific TM, Marietta, USA) at  $29^{\circ}\text{C}$  and 150 rpm. The initial sample 1 (leaves) after being lyophilized and ground weighed 19.5 g and the initial sample 2 (stems) weighed 15.6 g; this weight was brought to a 1:6 weight/volume with 96% ethanol.

The obtained extract was vacuum filtered using filter paper (Qual. Dia. 125 mm, BOECO, Germany) with the help of a funnel and vacuum pump (DOSIVAC, Buenos Aires, Argentina). Subsequently, the ethanol extract of *C. urens* was concentrated under reduced pressure in a rotary evaporator (IKA®RV10, Wilmington, USA) at 50 rpm with 150 mbar and  $40^{\circ}\text{C}$ . The concentrated extract was stored in amber jars at  $4^{\circ}\text{C}$  for later antifungal activity testing against *Colletotrichum* spp.

### Isolation and purification of *Colletotrichum* spp. strain

The fungus *Colletotrichum* spp. was isolated from a tomato fruit samples (*Lycopersicon esculentum*) exhibiting anthracnose symptoms. (Fig. 2).



**Figure 2. Tomato fruit (*Lycopersicon esculentum*) exhibiting post-harvest anthracnose symptoms.**

The fruits were sourced from the “Abastos” central market in Cucuta. The tomato samples were disinfected with 2% sodium hypochlorite for 5 min and subsequently rinsed with 70% ethanol for 3 min. The fruit explants were plated in triplicate on PDA (Merck) medium and incubated for 72 h at a temperature of 28°C. Upon observing fungal growth on the PDA medium, a single conidium was isolated under a microscope using a sterilized inoculation loop. This conidium was transferred to a fresh PDA plate to allow its growth. Once the conidium germinated and grew, the resulting colony was confirmed to have originated from a single conidium, ensuring a monoconidial culture. Successive re-plating was performed to further ensure a pure strain of the fungus of interest.

### Molecular identification of isolated and purified strains of *Colletotrichum* spp.

For the extraction of fungal DNA, two purified strains of the fungus were utilized. Our extraction protocol was adapted and based on the methodologies described previously (Martínez-Culebras, 1999; Alvarez *et al.*, 2004). The verification of *Colletotrichum* spp. genomic DNA was carried out using a 1% agarose gel, where the extracted genetic material from the C1 and C2 samples, corresponding to the fungal strains, was observed. DNA amplification was performed in a LTCC-48-101 gradient thermocycler (Labocon, Leicester, UK) with a final reaction volume of 25  $\mu$ L, using ITS 3 (5'-GCATCGATGAAGAACGCACC-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') oligonucleotides. The reaction mixture was prepared using Biobas® Taq polymerase (Ipswich, MA), following the manufacturer's instructions. Denaturation began with a temperature parameter of 95°C for 5 min, followed by denaturation at 95°C for 30 s, hybridization at a temperature of 52°C for 30 s, and final extension at 68°C for 2 min in 35 cycles.

The verification of the amplified products was carried out on 1% agarose gels, which were subsequently stained with Gelred® (Biotium, San Francisco, CA). The PCR product obtained was purified using the Bioline's Isolate II PCR and Gel Kit. The purified product was sent to MacroGen for sequencing. The nucleotide sequence obtained was compared with a search for homologous sequences using the BLAST algorithm, in the gene bank database of the NCBI (Benson *et al.*, 2005).

### Identification of secondary metabolites by gas chromatography-mass spectrometry (GC-MS)

The ethanolic extracts of *C. urens* (leaves and stems) were submitted to a specialized service for GC-MS analysis. While the exact sample preparation procedure is proprietary to the service provider, it generally involves derivatization or other sample preparation techniques to enhance the detectability of specific compounds such as fatty acids.

The chromatographic analysis was carried out using an AT6890 Plus series gas chromatograph equipped with an MSD 5973 mass spectrometric detector (Agilent Technologies, Santa Clara, USA) in full scan mode. The analysis utilized a DB-5MS column (5% phenyl-polymethylsiloxane, 60 m  $\times$  0.23 mm  $\times$  0.25  $\mu$ m). The injection was executed in Split mode (30:1) with the use of a Solid Phase Microextraction (SPME) device.

The identification of the compounds was facilitated by the Adams, Wiley, and NIST databases. Specific details on the detection algorithm or software used in the NIST database comparison might be proprietary to the service provider.

### Experimental design

The experimental design was structured around two experimental groups, each comprising eight treatments. These treatments involved dilutions of the extracts (extract-ethanol), a negative control using the antifungal agent Vitavax at a concentration of 24 mg mL<sup>-1</sup>, and a positive control with the PDA medium devoid of any added treatments. Each treatment was replicated in triplicate.

The primary variable measured during the study was the diameter of the fungal growth. Data analysis was conducted using SAS v 8, employing analysis of variance (ANOVA) to determine the significance of the observed differences among treatments. After observing significant differences in the ANOVA, multiple comparisons were conducted using Tukey's *post hoc* test to pinpoint specific differences between individual treatment groups.

The culmination of the experiment was marked by the complete growth of the fungal mycelium in the positive control Petri dish (Tab. 1).

**Table 1. Summary of treatments using various dilutions of *C. urens* extracts. The dilutions are specified in the format 'extract:ethanol'. Vitavax fungicide was used as a positive control.**

Treatment	Description
-Control	PDA – negative control
+Control	PDA + positive control Vitavax
1:0	PDA + Dilution 1:0
1:1	PDA + Dilution 1:1
1:2	PDA + Dilution 1:2
1:3	PDA + Dilution 1:3
1:4	PDA + Dilution 1:4
1:5	PDA + Dilution 1:5

## RESULTS

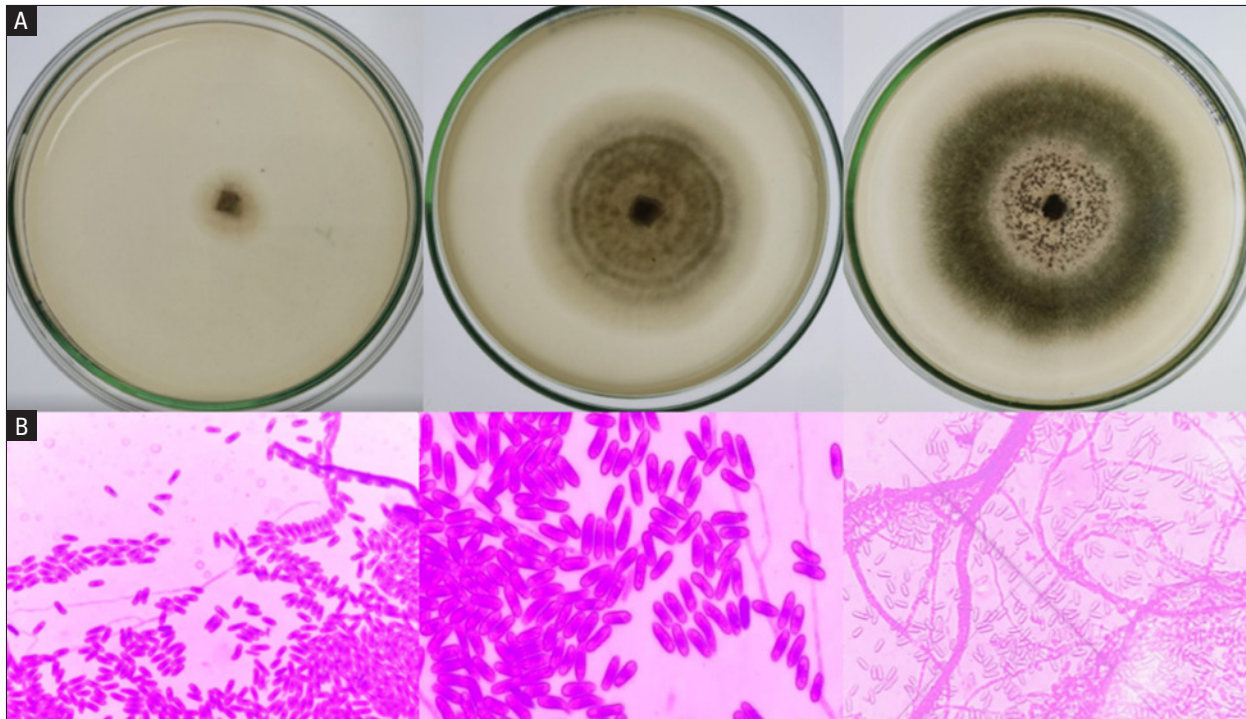
### Macroscopic and microscopic identification of *Colletotrichum* spp.

Once the disinfection and sowing process of tomato explants showing symptoms of *Colletotrichum*

infection was carried out, the macroscopic and microscopic identification of the fungus was performed using the lactophenol cotton blue staining technique. This method was used for the characterization of conidia and fungal mycelium with the aim of identifying the *Colletotrichum* spp. Genus, using Barnett and Hunter's key (Barnett and Hunter, 1986). The results provide substantial evidence that the fungus' growth observed on the culture plate, alongside the microscopic features of the hyphae and conidia, strongly corroborate the identification of the isolated fungus as *Colletotrichum* spp. (Fig. 3).

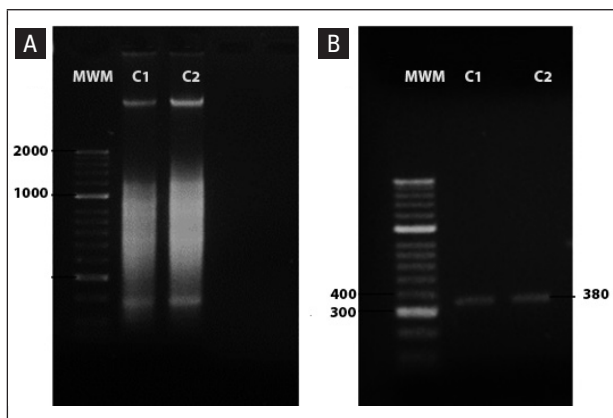
### Molecular identification of *Colletotrichum* spp.

The amplification of the genomic DNA of the fungus via PCR yielded a band approximately 380 bp in size (Fig. 4). Comparative analysis of the amplified DNA sequence from strain C1 corresponds to the ITS 1, 5.8S ribosomal gene, internal transcribed spacer 2, and the partial sequence of the 28S ribosomal gene across various isolates of *Colletotrichum* spp. The sequence from strain C1 displayed a 99.36% similarity and 98% coverage with *Colletotrichum boninense*,



**Figure 3. A) macroscopic identification of *Colletotrichum* spp. isolated from *Lycopersicon esculentum* on the third, sixth, and fifteenth day of incubation. B) microscopic characterization of *Colletotrichum* spp. strain C1 at 40× magnification. The fungal hyphae and conidia are distinctly observed.**

presenting an E-value of  $2e-155$ . However, no sequence was obtained from the C2 isolate, possibly due to inadequate purification of the DNA segment.



**Figure 4.** Genomic analysis of purified strains of *Colletotrichum* spp. **A.** Extraction of genomic DNA. **B.** Amplification of the ITS 3-4 intergenic region specific to *Colletotrichum* spp., displaying an expected amplified band of approximately 380 bp in size.

### Main compounds in the ethanolic extracts of *C. urens*

From the GC/MS analysis, several compounds were identified in the ethanolic extracts from the leaves and stems of *C. urens*. Among these compounds identified in the leaf extract are n-hexadecanoic acid, nonadecanoic acid, ethyl ester, phytol, and 9,12-octadecadienoic acid, ethyl ester. The stem extract analysis revealed the presence of certain fatty acids and triterpenoids. It's important to note that these are not the only components, but those detected by our GC/MS analysis. Further analyses, such as HPLC, could reveal additional compounds that might also contribute to the antifungal activity (Tab. 2).

This group of compounds has previously been identified with various biological properties, highlighting antimicrobial characteristics. n-Hexadecanoic acid has been cataloged as a defense to prevent infections. Similarly, the presence of phytol, a diterpenoid, has been highlighted as potential for a wide range of applications in the pharmaceutical and biotechnological industry.

Further insight into the chemical constituents of the ethanolic extracts from *C. urens* can be gleaned from the chromatograms presented. For a detailed visual representation of the peaks corresponding to the

**Table 2.** Compounds identified by GC-MS analysis in the ethanol extract (leaves and stem) of *C. urens* L.

T <sub>R</sub> (Min)	Compounds	Relative amount GC
<b>Leaves</b>		
37,117	n-Hexadecanoic acid	99%
37,8133	Nonadecanoic acid, ethyl ester	94%
41,8446	Phytol	76%
43,2457	9,12-Octadecadienoic acid, ethyl ester	99%
<b>Stem</b>		
37,091	n-Hexadecanoic acid	97%
49,6577	Squalene	91%

primary metabolites identified in both the leaves and stems, refer to figure 5.

### Efficacy of *C. urens* ethanolic extracts against *Colletotrichum* spp.

Both the ethanolic leaf and stem extracts of *C. urens* were evaluated for their antifungal activity against *Colletotrichum* spp. at six different dilutions (1:0 to 1:5). The controls used for the experiment were water as a negative control, indicating normal fungal growth, and the antifungal Vitavax (+control), indicative of inhibited fungal growth. Remarkably, both the leaf and stem extracts (Fig. 6) displayed equivalent antifungal activity to the +control, successfully inhibiting the growth of *Colletotrichum* spp. In stark contrast, the fungus experienced normal growth in the presence of the -control, confirming the antifungal activity of the *C. urens* extracts (Fig. 6).

## DISCUSSION

*Colletotrichum* spp. is a ubiquitous phytopathogenic fungus that inflicts disease across a wide range of plant species, notably impacting productivity and fruit quality. Given the rising demand for sustainable and environmentally friendly control strategies, this study explored the potential of natural plant extracts as antifungal agents. We specifically examined *C. urens*, a plant species that has demonstrated potential in earlier studies (Chaves-Bedoya and Ortiz-Rojas, 2022). Its effectiveness against *Colletotrichum* spp. was evaluated using various concentrations of leaf and stem extracts.

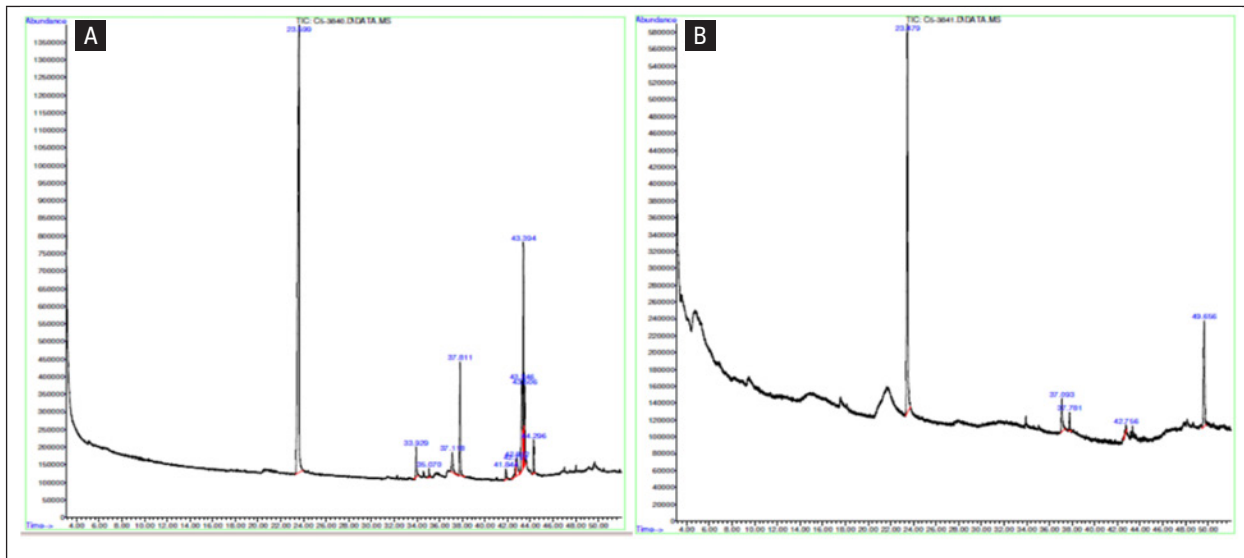


Figure 5. Chromatograms of the ethanolic extract from *C. urens*. A) leaves: peaks corresponding to the primary identified metabolites are observed. B) Stems: peaks corresponding to the primary identified metabolites are observed.

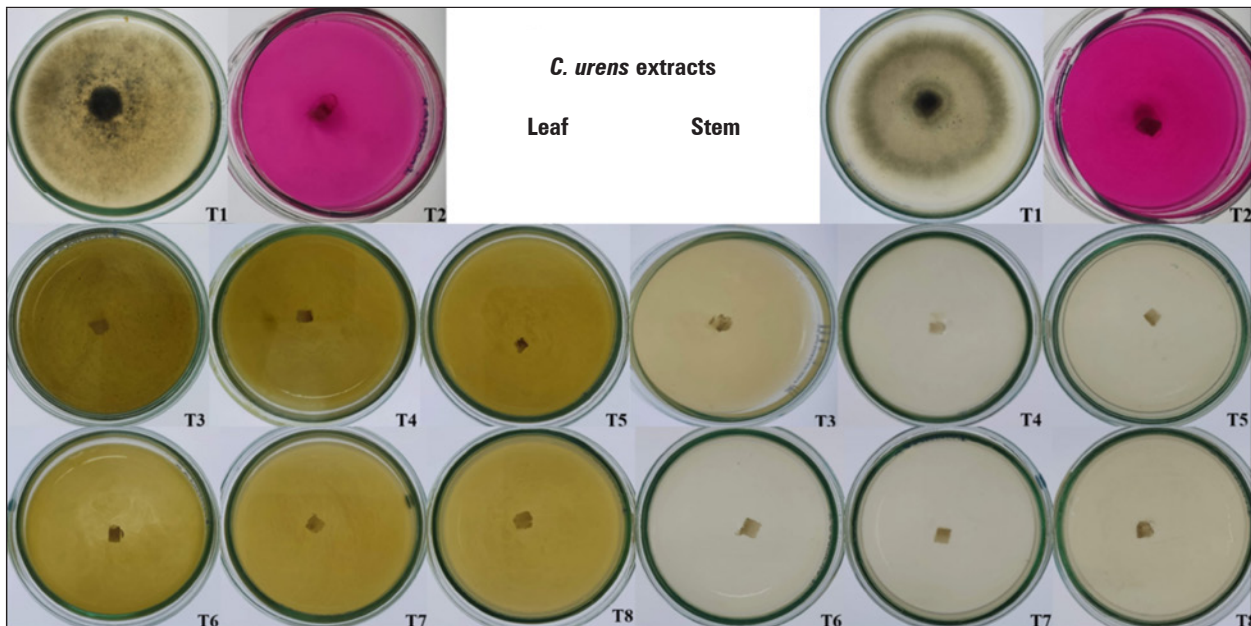


Figure 6. Inhibitory effect of *C. urens* leaf and stem extracts against *Colletotrichum* spp. The figure illustrates the inhibitory effects of both leaf and stem extracts of *C. urens* on the growth of *Colletotrichum* spp. In each respective petri dish, it shows a comparison between the control growth and the inhibited fungal growth following the application of the extracts. The differences in fungal growth between the control and extract-treated dishes highlight the potential of *C. urens* extracts as a natural fungicide. T1 negative control, T2 positive control (Vitavax), T3 dilution 1:0, T4 dilution 1:1, T5 dilution 1:2, T6 dilution 1:3, T7 dilution 1:4, T8 dilution 1:5.



An analysis of variance (ANOVA) was conducted to assess the impact of *C. urens* leaf and stem extracts on inhibiting *Colletotrichum* spp. growth. Several factors were contemplated in this study, which included the type of extract (leaf or stem), the extract concentration, and the repetition of the experiment. The analysis disclosed that both the type of extract and its concentration significantly affected the inhibition of fungal mycelial growth.

According to the ANOVA outcomes, *C. urens* extracts are successful in stymieing the growth of *Colletotrichum*, and this efficacy does not show any significant fluctuation between leaf and stem extracts or across various extract concentrations. The GC-MS analysis of the ethanolic extracts unveiled the presence of secondary metabolites, including terpenoid-type compounds. These constitute the most significant class of natural products used across diverse sectors like the food industry, pharmaceuticals, chemicals, and lately, they have also been exploited in the development of biofuel products. The presence of these critical compounds makes plants a valuable source for the discovery of novel products with medicinal value and drug development (Chaves-Bedoya and Ortiz-Rojas, 2022).

Recent studies have demonstrated the effectiveness of certain terpenes such as phytol, which showcases antimicrobial properties, specifically an antifungal effect against *Candida* spp. from the vegetable extract of *Syzygium cumini* (L.) (Figueiredo *et al.*, 2021). Also, numerous investigations have verified the antimicrobial capability of fatty acids such as palmitic acid (n-hexadecanoic acid) and its derivatives, which are deemed as the most potent defense mechanism of certain organisms to prevent infections. Palmitic acid has shown significant inhibition results against various *Candida* spp. species (Quiroz-Lobo *et al.*, 2022). Based on these findings, the major compounds identified in the ethanolic extracts of *C. urens* could potentially contribute to the biological activity against the phytopathogen *Colletotrichum* spp.

The results show that the vegetable extract dilutions from *C. urens*, both from leaves and stems, effectively inhibited the growth of *Colletotrichum* spp. across all tested concentrations from treatment 1:0 to treatment 1:5. The fungal radial growth, measured from the center of the inoculum to the growing edge, was only 1 mm under these conditions, compared to the 55 mm observed in the positive control corresponding to treatment negative control. This significant

finding suggests that these extracts may harbor potent antifungal properties. In other words, no significant differences were observed between the efficacy of leaf and stem extracts based on these data. These findings support the idea that *C. urens* extracts could serve as an effective biological antifungal alternative or plant protection agent for controlling fungal diseases across diverse plant species. This indicates that the antifungal compounds present in this plant could be distributed both in leaves and stems, a valuable observation for future investigations of these extracts as potential antifungal agents.

Compared to the positive control (Vitavax), the extracts showed similar efficacy in restraining the mycelial growth of the fungus to 1 mm. This is encouraging as it suggests that *C. urens* extracts could be as potent as existing commercial fungicides. With regard to the efficacy of different dilutions, there was no significant variation in the ability to inhibit fungal growth across multiple extract concentrations. Nevertheless, conducting further tests would help confirm these results and identify if an optimal concentration exists. Further, it is crucial to identify the specific antifungal compounds in the extracts and investigate their potential applications in practical scenarios, such as treating plant fungal diseases.

In addition to the major compounds identified in the ethanolic extracts of *C. urens*, it is critical to note the potential of unexplored or unidentified compounds that may contribute to the observed antifungal activity. The synergistic effect of various compounds could play a pivotal role in enhancing the efficacy against *Colletotrichum* spp. For instance, recent studies have highlighted the importance of plant synergism where a combination of compounds can result in enhanced antimicrobial activity (Vaou *et al.*, 2022).

Moreover, the potential of these extracts extends beyond being merely antifungal agents. Plant-based extracts have been documented for their antioxidative, anti-inflammatory, anticancer, and various other medicinal properties (Yuan *et al.*, 2016). The presence of secondary metabolites like terpenes and fatty acids in the extracts from *C. urens* could potentially have similar multi-faceted health and medicinal benefits, thus warranting further research in this direction.

Finally, while our findings suggest promising potential of *C. urens* extracts in controlling fungal growth, it is also essential to consider the practicality and implications of these findings. Factors like the availability

and growth rate of *C. urens*, the scalability of extract production, and potential environmental impacts of large-scale implementation need to be carefully evaluated. The safety and potential side effects, especially if applied in a food crop context, must also be considered. In essence, while laboratory results are indeed promising, translating these findings into effective, safe, and environmentally friendly solutions requires comprehensive multidisciplinary research.

Therefore, the results of this study not only underscore the potential use of *C. urens* extracts in managing phytopathogenic fungi but also open avenues for broader explorations into their biochemical properties and potential applications in diverse sectors, including medicine, agriculture, and the food industry.

## CONCLUSION

Through this study, we not only accomplished the morphological and molecular characterization of the *Colletotrichum* spp. fungus isolated from *Lycopersicon esculentum* fruits with disease symptoms, but we also shed light on the *in vitro* efficacy of *C. urens* extracts. Our observations suggest that all tested concentrations of these extracts were as potent as the commercial fungicide Vitavax in inhibiting the mycelial growth of *Colletotrichum* spp.

We further discerned the presence of key compounds such as terpenes and fatty acids in the extracts, which we posit, could be instrumental in the antifungal properties exhibited. Our findings thus bolster the hypothesis that *C. urens* extracts could serve as a viable and sustainable alternative to commercial fungicides for managing *Colletotrichum* spp. growth.

While our research affirms the antifungal potential of *C. urens* extracts, it also opens avenues for future studies. Deeper investigations could focus on identifying the precise antifungal compounds and understanding their mechanisms of action. Furthermore, field studies would be beneficial to test the practical applicability and environmental impact of using these plant extracts as bio-fungicides. Our study underscores the importance of embracing eco-friendly approaches to agriculture, fostering a balance between productivity and sustainability.

## Declaration of competing

The authors declare that there are no conflicts of interest.

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