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Evaluation and antifungal potential of polyphenolic compounds from Dalea carthagenensis for the control of Rhizopus stolonifer in jackfruit

Evaluación y potencial antifúngico de los compuestos polifenólicos de Dalea carthagenensis para el control de Rhizopus stolonifer en yaca

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ABSTRACT

The soft rot caused by Rhizopus stolonifer is the most important disease in jackfruits. Currently, the control of this phytopathogen is achieved with toxic fungicides. Due to this, the control of R. stolonifer is sought through natural alternatives such as plant extracts. This study aimed to identify the compounds in Dalea carthagenensis extract and evaluate its antifungal potential against the postharvest phytopathogen R. stolonifer. The antioxidant activity of the extract and the in vitro activity against R. stolonifer was evaluated. The phytochemical compounds were identified by HPLC-UV-Vis and the in vivo antifungal activity was assessed against R. stolonifer in jackfruit. The extract presented an antioxidant activity of 48.34 ± 1.3 mg TE/g dw and a total phenolic content of 52.23 ± 0.57 mg GAE/g dw. In vitro, R. stolonifer was inhibited between 27.46-79.71 % and presented a minimum inhibitory concentration of 11.08 mg/mL. D. carthagenensis extract, in which gallic acid and vanillin were mainly identified, reduced the severity of soft rot by 59 % in jackfruits. In conclusion, D. carthagenensis extract is a promising alternative for managing soft rot disease in jackfruit at the postharvest stage.

KEY WORDS: Rhizopus stolonifer, Jackfruit, Dalea carthagenensis, Plant extract, Postharvest.

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RESUMEN

La pudrición blanda causada por Rhizopus stolonifer es la enfermedad más importante en el fruto de yaca. Actualmente, el control de este fitopatógeno se logra con fungicidas tóxicos. Debido a esto, se busca el control de R. stolonifer a través de alternativas naturales como los extractos de plantas. Este estudio tuvo como objetivo identificar los compuestos del extracto de Dalea carthagenensis y evaluar su potencial antifúngico contra el fitopatógeno poscosecha R. stolonifer. Se evaluó la actividad antioxidante del extracto y la actividad in vitro contra R. stolonifer. Los compuestos fitoquímicos se identificaron mediante HPLC-UV-Vis y se evaluó la actividad antifúngica in vivo contra R. stolonifer en yaca. El extracto presentó una actividad antioxidante de 48.34 ± 1.3 mg TE/g dw y un contenido fenólico total de 52.23 ± 0.57 mg GAE/g dw. In vitro, R. stolonifer fue inhibido entre 27.46-79.71 % y presentó una concentración mínima inhibitoria de 11.08 mg/mL. El extracto de D. carthagenensis, en el que se identificaron principalmente el ácido gálico y la vainillina, redujo la severidad de la enfermedad en 59 % en los frutos de yaca. En conclusión, el extracto de D. carthagenensis es una alternativa prometedora para el manejo de la pudrición blanda en yaca en etapa poscosecha.

PALABRAS CLAVE: Rhizopus stolonifer, Yaca, Dalea carthagenensis, Extracto de planta, Poscosecha

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.), a member of the Moraceae family, is an important tropical fruit cultivated in various regions, including China, India, Malaysia, Bangladesh, and Mexico. Its cultivation in Mexico commenced in 1960 and it has expanded to approximately 1751 hectares across six states. Each year, Mexico produces around 23,995 tons of jackfruit, with Nayarit emerging as the primary contributor, responsible for approximately 93 % of the total production (Barros-Castillo *et al*., 2021).

Jackfruit is an abundant source of dietary fiber, vitamins, antioxidants, and laxative properties (Ghosh *et al*., 2015). However, during the pre and postharvest stages, jackfruits are susceptible to attack by phytopathogenic fungi, such as *Rhizopus stolonifer*, causing substantial losses estimated at between 30 to 50 % of production. This not only affects the nutritional quality of the fruit but also causes damage to the fruit itself (Covarrubias-Rivera *et al*., 2022).

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The symptoms of *R. stolonifer* infection include watery areas quickly covered by coarse, and gray hairy mycelia forming a mass of black sporangia at their tips. Infection typically occurs during harvest and handling. Given *R. stolonifer's* ability to infect a wide range of hosts and its fast penetration and colonization, it has become a significant target for control. Synthetic fungicides, such as benomyl, imazalil, propiconazole, thiabendazole, and thiocarbamates, are commonly employed to control *R. stolonifer*. However, their excessive, irrational, and indiscriminate use raises concerns about consumer safety and environmental threats (Bautista-Baños *et al*., 2014). This situation underscores the importance of exploring alternative methods for controlling fungal infections in jackfruit. In line with this need, the European Green Deal and the United Nations 2030 Agenda have set ambitious goals to reduce by 50 % the use of chemical fungicides by 2030, emphasizing the urgency of finding sustainable and eco-friendly solutions to fungal diseases in fruits (Lázaro *et al*., 2021). In response, recent research has focused on exploring natural alternatives to control post-harvest diseases, leading to increased interest in plant extracts and their potential benefits. Researchers have recognized the antifungal activity of plant extracts against *Rhizopus*, and their phenolic compounds (Vilchis-Gómez *et al*., 2024) such as *Barringtonia racemosa* L (Hussin *et al*., 2009), *Malva sylvestris* L (Zohra *et al*., 2013), *Melaleuca styphelioides* leaves (Laribi *et al*., 2021), among others.

In this context, *Dalea carthagenensis*, belonging to the Fabaceae family and distributed across Central America, northern South America, and the Antilles, has garnered attention due to its content of phenolic compounds such as flavonoids, flavones, tannins, chalcones, and coumarins (Montes-de-Oca-Márquez *et al*., 2017). Regarding the mechanism of action concerning the antifungal properties of phytochemicals, three primary pathways have been reported: inducing cytotoxic effects in fungi that disturb the integrity and functions of the cell membrane; blocking crucial genes and enzymes involved in fungal biosynthesis pathways; and interfering with cellular compartments like mitochondria, causing imbalances in osmotic pressure and redox reactions (Chen *et al*., 2021; Hu et al., 2021; Torgbo *et al*., 2022). Therefore, this research could contribute significantly to reducing postharvest losses of jackfruit by offering a sustainable method to control *R. stolonifer*. By taking advantage of the antifungal properties of the High-Value Biological Compounds (HVBCs) present in *D. carthagenensis*, this extract could provide a natural and effective form to preserve the quality and extend the shelf life of jackfruit, avoid economic losses, and enhance food security. Hence, this research aims to identify the HVBCs present in *D. carthagenensis*, explore its antifungal capacity, and propose a natural alternative for combating soft rot disease caused by *R. stolonifer* in jackfruit.

Material and Methods

Plant materials

D. carthagenensis leaves were collected from Puebla, Mexico (18º 12' and 18º 14'N; 97º 07' and 97º 09' W; altitude 957 masl). The leaves were washed with water, dried at 60 °C for 24 h in a convection oven (Model HS60, Novatech Ltd, Guadalajara, Mexico), and then pulverized with an electric grinder (Model NB-201, NutriBullet®, Los Angeles, USA). The powder was sieved

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through a 150 µm mesh and kept at 25 °C until use.

Healthy, homogeneous (weight: 10 ± 1 kg, length: 30 ± 5 cm), in physiological maturity stage (light green color), and undamaged jackfruits (*A. heterophyllus* Lam. cv. Romina) were harvested from a commercial orchard located in Zacualpan, Nayarit, Mexico (21º 15′N 105º10′O). The fruits were transported in plastic boxes and stored at 4 ºC for 24 h until use. Jackfruits were individually washed with water and immersed in 2 % (v/v) NaClO solution for 3 min, rinsed with sterile purified water, and dried at 25 °C for 30 min.

Obtention of the *D. carthagenensis* extract by ultrasound-assisted extraction (UAE)

D. carthagenensis extract was obtained following the methodology proposed by Vázquez-González *et al*. (2020) with some modifications, briefly, a mixture of ethanol:water (80:20 v/v) was used as extraction solvent combined with the sample in a ratio of 1:10 (g dry sample:mL solvent). The ground material was mixed with the solvent and cooled at 10 °C to maintain the sample at 25 °C. The HVBCs were extracted by using an ultrasonic bath (Model CD4820, Viewlight®, Guangdong, China). The sample was sonicated for 30 min at 42 kHz. Afterward, it was filtered using Whatman No.1 filter paper and the solvent was evaporated (Model RV 10 basic S1, IKA, Staufen, DEU) at 45 °C and -90 kPa. The concentrated extract was kept at 4 °C until used.

Antioxidant capacity

$ABTS^+$ Radical Scavenging Activity (RSA)

The $ABTS^+$ RSA was performed following the methodology proposed by Vilchis-Gómez *et al*. (2024) by using a stock solution of $ABTS^+$ (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonate) (Sigma-Aldrich, St. Louis, MO, USA), at 7 mM in potassium persulfate (2.45 mM) (Sigma-Aldrich). The solution was kept in darkness at 25 °C for 15 h. The stock solution was diluted in distilled water and adjusted to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 50 µL of the extract (0.5 – 6 mg/mL) were mixed with 950 µL of $ABTS^+$ using a vortex for 10 s. Succeeding a 7-minute interval, the absorbance was measured at 734 nm with a spectrophotometer (Model Cary 50 Bio UV–Visible, Varian, Mulgrave, Australia). The $ABTS^+$ RSA was calculated (Eq. 1).

$$ABTS^+\ RSA\,(\%) = \frac{Acontrol - Amuestra}{Acontrol} \times 100$$

Equation 1.

Where: Acontrol represents the absorbance of the $ABTS^+$ diluted solution and Asample represents the absorbance of $ABTS^+$ reacting with extract sample.

Curves representing the percentage of RSA on the y-axis versus the concentration of the sample (mg/mL) on the x-axis were plotted for each sample. The results were expressed in mg of Trolox equivalents per gram of dry weight (mg TE/g dw). The experiment was performed in

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triplicate and was repeated three times.

Determination of IC50 values

The IC50 value was defined as the concentration of extract (mg/mL) required to achieve 50 % of antioxidant activity and was determined from the linear regression equation of curves representing antioxidant capacity (%) versus sample concentration (mg/mL) according to Calderón-Chiu *et al*. (2021).

Determination of soluble phenolic content (SPC)

To determine the SPC, 50 µL of the extract or the standard solution was mixed with 1 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent (Sigma-Aldrich). Then, the mixture was supplemented with 2.5 mL of a 10 % (w/v) Na_2CO_3 solution which was gradually prepared to avoid agglomeration, and incubated in complete darkness for 20 min at 25 °C. The absorbance was measured at 735 nm using a spectrophotometer. For the blank, distilled water was used. A standard curve was prepared using gallic acid (Sigma-Aldrich) (0.025–0.5 mg/mL; y= 1.6125x-0.0297; r^2= 0.9993; y represents absorbance, and x the solution ion concentration) (Liu *et al*., 2009). SPC results were expressed as mg equivalents of gallic acid per gram of dry weight (mg GAE/g dw). The experiment was performed in triplicate and was repeated three times.

Evaluation of the antifungal activity of the *D. carthagenensis* extract

Preparation of the fungal pathogen

The phytopathogenic fungus evaluated in this study was previously isolated and phylogenetically characterized. *R. stolonifer* ATR1 (Accession number: OP683835) from jackfruit (*A. heterophyllus* L.) (Ayón-Macias *et al*., 2023) belongs to the fungal collection of the Food Microbiology Laboratory of the Instituto Tecnológico de Tepic. *R. stolonifer* ATR1 was cryopreserved in glycerol 80 % (v/v) at -80 °C until its use. The fungus was cultivated in Petri dishes with Potato Dextrose Agar (PDA) (Becton Dickinson & Co., Estado de México, Mexico) and incubated at 28 °C for 3 days. The spore suspension was prepared as follows: 15 mL of 0.85 % NaCl sterile solution were added to the culture and scraped with a sterile loop. The liquid was filtered and collected in a conical tube. The spore concentration was adjusted to 1 × 10^5 spores/mL with a hemocytometer (LO-Laboroptik Ltd, Lancing, UK) as described in González-Gutiérrez *et al*. (2023).

In vitro antifungal activity

The *in vitro* antifungal activity was carried out according to Covarrubias-Rivera *et al*. (2022). Different concentrations of the *D. carthagenensis* extract (1, 3, 5, 7, and 9 mg/mL) were individually mixed with PDA and then poured into Petri dishes. The agar was allowed to solidify and dry at 25 °C for 1 h. Each plate was perforated in the center and then, 20 µL of the spore suspension (1 × 10^5 spores/mL) of *R. stolonifer* ATR1 were inoculated. The positive control consisted of PDA Petri dishes with the pathogenic fungus. The Petri dishes were incubated at 28 °C for eight days.

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At the end of the experiment, the fungal colony was measured and the mycelial growth inhibition was calculated (Eq. 2.). To calculate the minimum inhibitory concentration (MIC), the values of the linear equation were obtained by plotting the mycelial growth diameter against the extract concentration. A unifactorial statistical design was applied and the response variable evaluated was the percentage of *in vitro* inhibition. The experiment consisted of three repetitions and five replicates per treatment.

$$Inhibición\ del\ crecimiento\ micelial\ (\%) = \left[\frac{CP-ET}{CP}\right] \times 100$$

Equation 2.

Where: CP (control pathogen) was the mean diameter of the fungus without extract and ET (extract treatment) was the mean diameter of the fungus in the presence of *D. carthagenensis* extract.

In vivo antifungal activity

The *in vivo* antifungal activity of the *D. carthagenensis* extract was assessed against *R. stolonifer* in jackfruits cv. Romina. Briefly, three holes per jackfruit were made with a sterile bodkin (5 mm deep and 5 mm wide). Each hole was sprayed with 2 mL of the extract (MIC 11.08 mg/mL) dissolved in sterile distilled water. The jackfruits were left to air-dry for 1 h. After that, 15 µL of *R. stolonifer* spore suspension (1×10^5 spores/mL) was inoculated into each hole (Ayón-Macias *et al*., 2023). The control treatment was inoculated only with spore suspension. The fruits were stored in a camera (Model CA-550, Novatech Ltd, Kingwood, USA) at 25 ± 1 °C and 95 % relative humidity for five days (González-Gutiérrez *et al*., 2024). At the end of the experiment, the symptom severity was evaluated using a category scale from 0 to 4, where 0= no visible rot; 1= 1 to 25 %; 2= 25 to 50 %; 3= 50 to 75 %; and 4= >75 % of the jackfruit surface with soft rot (Guarnaccia *et al*., 2016). In addition, the lesion diameter produced by *R. stolonifer* was measured to determine the severity (mm), and the disease incidence was calculated (Eq. 3). A univariate statistical design was applied and the response variable evaluated was the disease incidence. The experiment consisted of three repetitions with three replicates per treatment and five jackfruits per replicate.

$$Incidencia\ de\ la\ enfermedad\ (\%) = \frac{Numero\ de\ frutos\ infectados}{Frutos\ totales}\ (100)$$

Equation 3.

Polyphenol profile by HPLC-UV-Vis

The extract was evaporated until it was dry in a rotary evaporator at 50 °C. The dried residues were dissolved in a 2 mL mobile phase. After the sample was filtrated through a 0.45 µm PTFE syringe filter (Millipore, Milford, USA) directly into sample vials (Marcillo-Parra *et al*., 2021).

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The identification of HVBCs from *D. carthagenensis* was performed according to Lerma-Torres *et al*. (2019) using an HPLC-UV-Vis (HPLC Model 210, UV-Vis detector Model 320, Varian ProStar, USA). The separation was carried out on a Shimadzu CLC-ODS (C18) column (25 cm × 4.6 mm × 5 µm) at 25 °C. The eluent phase consisted of solvent A (3 % acetic acid in water) and solvent B (acetonitrile). The process conditions were 10 µL injection, 167 bars pressure, a flow rate of 1 mL/min, run time of 16 min. The program was carried out in gradient starting with 90 % eluent A and 10 % B, then 6 min from 20 % A and 80 % B, and finally 10 min with 90 % A and 10 % eluent B. Detection wavelength was 254 nm. The identification of the HVBCs was achieved by comparing the retention time of the peaks with authentic standards.

Statistical Analysis

The results were presented as the mean ± standard deviation of three repetitions. Statistical analysis was performed using STATISTICA software version 12.0 for Windows (StatSoft, Inc.). The data underwent a one-way analysis of variance. To compare means, the post-hoc least significant difference (LSD) Fisher test ($p \leq 0.05$) was employed. Microsoft Office Excel 2019 (Microsoft Corporation, Redmond, CA, USA) was used to calculate the MIC values through *in vitro* antifungal activity.

Results and Discussion

Antioxidant capacity of *D. carthagenensis* extract

The antioxidant activity of *D. carthagenensis* extract quantified by $ABTS^{+}$ was 48.34 ± 1.3 mg TE/g dw (Table 1). The antioxidant activity of *D. carthagenensis* is conferred by the chemical structure of the phenolic compounds (hydroxylated aromatic ring, carboxylic group, or methoxyl group) (El-Nagar *et al*., 2020) that allow the stabilization and relocation of unpaired electrons, facilitating the donation of hydrogen atoms and electrons from their hydroxyl groups (Chaves *et al*., 2020). For this parameter, as the value increases, the antioxidant activity increases. Despite this, lower values have been reported in other studies, such as extracts of *Terminalia arjuna* (6.99 ± 0.31), *Glycyrrhiza glabra* (3.34 ± 0.14), and *Vitex negundo* (2.53 ± 0.12 mg TE/g dw) (Rajurkar & Hande, 2011). In addition, the extract concentration to inhibit 50 % of $ABTS^{+}$ radicals was 0.011 ± 0.003 mg/mL (Table 1). The activity against the DPPH radical was relevant compared to others, for example, *Adelia ricinella* extract with IC50= 0.45 mg/mL (Berenguer-Rivas *et al*., 2018). This evidences that *D. carthagenensis* has greater antioxidant activity since less extract is needed to inhibit radical formation.

On the other hand, *D. carthagenensis* extract presented 52.23 ± 0.57 mg GAE/g dw (Table 1). In contrast to other studies, *D. carthagenensis* showed higher SPC than the ethanolic extract of jackfruit leaves (36 ± 1.75) (Vázquez-González *et al*., 2020), than the medicinal plant *Acacia nilotica* (15.88 ± 0.54) (Rajurkar & Hande, 2011) and than the *Colocasia esculenta* extract (15 mg GAE/g dw) (Eleazu, 2016). The differences in the SPC vary depending on the plant species, plant tissue, developmental stage, and environmental factors, such as temperature, water stress,

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and light conditions (Chaves *et al*., 2020). The result for *D. carthagenensis* is acceptable since a high SPC leads to a great antioxidant potential of the extract. The determination of the antioxidant activity of *D. carthagenensis* could contribute to revealing the value of this species as a new source of antioxidant compounds.

Table 1. Antioxidant activity by ABTS, IC50, and total phenolic content of D. carthagenensis extract.

Properties	**Ethanolic extract**
	D. carthagenensis
ABTS+ (mg TE/g dw)	48.34 ± 1.3
IC50 radical ABTS+ (mg/mL)	0.011 ± 0.003
Soluble phenolic content (mg GAE/g dw)	52.23 ± 0.57

Source: Own elaboration based on results.

In vitro evaluation of *D. carthagenensis* extract against *R. stolonifer*

D. carthagenensis extract at concentrations lower than 5 mg/mL moderately controlled the mycelial growth of *R. stolonifer*. The antifungal activity increased as the extract concentration increased ($p < 0.05$) (Figure 1). *R. stolonifer* was inhibited between 27.46 - 79.71 % (Figure 1). These results showed more effectiveness than Lamiaceae herbal extracts with inhibition of *R. stolonifer* by 36.71 % (López *et al*., 2007).

The extract effectiveness in the inhibition of different fungi is attributed to differences in their cell wall composition (Covarrubias-Rivera *et al*., 2022), specific characteristics of the pathogens, such as growth rate, genes associated with pathogenic factors, among others (Yang *et al*., 2019), and to the total phenol content (Chaves *et al*., 2020). Phenolic compounds in plant extracts play a fundamental role in antioxidant and antifungal activities. These compounds can generally act through four possible mechanisms: interference in cellular structure and cellular biosynthesis, inhibition of the energy mechanism, and multisite activity (Aguilar-Veloz *et al*., 2020).

In this study, from 7 mg/mL of the extract, *R. stolonifer* presented important *in vitro* sensitivity (> 65 % mycelial growth inhibition). Based on the MIC result (11.08 mg/mL), a total inhibition of *R. stolonifer* was achieved. The R^2 coefficient was 0.9484, which was properly fitted with a value greater than 0.8 (Table 2). Considering that the effectiveness depends on the extract concentration, as well as the susceptibility and fungus characteristics, the antifungal effect of *D. carthagenensis* extract was evaluated in jackfruits against *R. stolonifer*.

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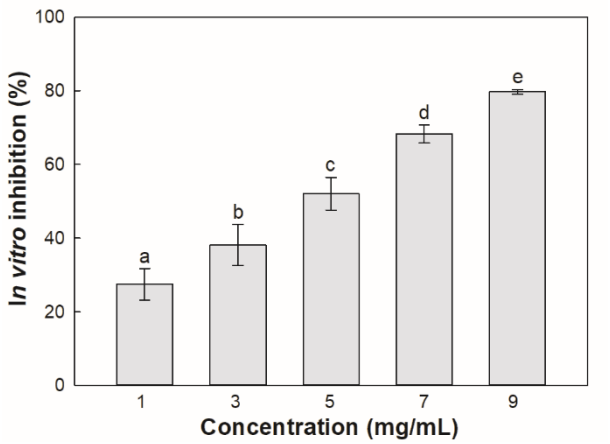

Figure 1. *In vitro* inhibition of *R. stolonifer* with *D. carthagenensis* extract. Data are the means and the vertical lines represent the standard deviation. The mean values with the same lowercase letter indicate that there is no significant difference between the treatments according to the Fisher LSD test ($p < 0.05$). Source: Own elaboration based on results.

Table 2. Minimum inhibitory concentration (MIC) of R. stolonifer with D. carthagenensis extract.

Phytopathogenic fungus	Parameter	Regression Equation	R^2	MIC (mg/mL)	In vitro inhibition (%)
R. stolonifer	φ Max	y= -0.6559x + 7.2662	0.9484	11.08	100 ± 0.0

Source: Own elaboration based on results.

Identification of the HVBCs of *D. carthagenensis* extract by HPLC

Seven HVBCs from *D. carthagenensis* extract were identified by HPLC-UV-Vis. Gallic acid and vanillin were the major compounds in *D. carthagenensis* leaves with a concentration in the extract of 10.03 and 2.70 mg/mL, respectively (Table 3). The retention time of all compounds coincided with the standards. These HVBCs have been identified in diverse plant extracts of *Cymbopogon citratus*, *Tinospora cordifolia*, *Mentha arvensis*, *Camellia sinensis*, *Foeniculum vulgare*, *Trachyspermum ammi* (Mueed *et al*., 2023), *A. heterophyllus* (Vázquez-González *et al*., 2020), and *Randia monantha* (Vilchis-Gómez *et al*., 2024). Likewise in fruits and vegetables such as mango fruit (Kumar *et al*., 2021), cabbage, celery, spinach, lettuce, broccoli, tomato, apple, and peach (Sakakibara *et al*., 2003), among others.

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This is the first study to report HVBCs of *D. carthagenensis*. This research can help to define the possible pharmacological properties, the value as a source of antioxidants, and the antifungal activity of the extract. In previous studies, the specific antifungal mechanism of the identified compounds has been elucidated (Table 3). On this basis, identifying and understanding the action mechanism of phenolic structures is important to design and develop effective and natural strategies for the control of fungal diseases, as is the case of the *in vivo* application of the extract against *R. stolonifer* in jackfruit during the postharvest stage.

Jackfruits stored at 25 ± 1 °C showed disease symptoms after 2 days. At the end of the test, the fruits treated with *D. carthagenensis* extract and control fruits presented 100 % incidence ($p > 0.05$). However, the lesion diameter of treated fruits (5.0 ± 0.44 cm) was 7.2 cm smaller than the control (12.2 ± 1.01 cm) (Figure 2a). Moreover, according to the severity scale, treated and control fruits were categorized between 1-25 % and 50-75 % of soft rot, respectively, (Figure 2b). *D. carthagenensis* extract reduced the severity of soft rot by 59 % and the disease signs were manifested as brown, watery, and soft areas covered by gray hairy mycelia and a mass of black sporangia (Figure 2c).

This study confirmed, for the first time, the *in vitro* and *in vivo* antifungal activity of the *D. carthagenensis* extract against *R. stolonifer*. Considering that gallic acid and vanillin are the main HVBCs in the extract, this behavior about the antifungal activity is mainly given by these compounds. In this way, the gallic acid can bind to ergosterol (a component of fungal cell membranes) and cause membrane permeability and inhibition of the enzymes involved in the ergosterol synthesis (Li *et al*., 2017; Carvalho *et al*., 2018). Vanillin may disrupt the fungal cell membrane and produce a leak of ions in the cells, it can affect the homeostasis of the intra and extracellular membranes and generates oxidative bursts that attack unsaturated fatty acids of the membrane (Li *et al*., 2021; 2022).

On the other hand, caffeic acid, chlorogenic acid, vanillic acid, scopoletin, and mangiferin, present in lower concentrations, can contribute to the inhibition of *R. stolonifer* and other pathogenic fungi. Some examples are *Candida auris* (Possamai-Rossatto *et al*., 2021), *Fusarium solani* (Martínez *et al*., 2017), *Sclerotium rolfsii* (Yousaf *et al*., 2023), *C. gloeosporioides* and *P. digitatum* (Vilchis-Gómez *et al*., 2024), respectively, for each compound. These chemical compounds can cause fungal cell permeability, alteration of cellular metabolism, damage to genetic material, and inhibition of protein synthesis (Table 3). These mechanisms could impact the reduction of spore germination and the ability of *R. stolonifer* to form appressoria, thus affecting the life cycle and colonization strategy of the fungus.

In vivo evaluation of *D. carthagenensis* extract against *R. stolonifer* in jackfruit

Although considerable natural strategies have been applied against *R. stolonifer*, our findings showed that *D. carthagenensis* extract only decreased the severity of the soft rot in jackfruits. Hence, the effectiveness of antioxidants is influenced by factors such as concentration, structural characteristics, temperature, and physical state of the system (Shahidi & Zhong, 2015). For this reason, to guarantee higher bioactivity and stability of the HVBCs during the *in vivo* test,

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it is necessary to develop extract application strategies such as microcapsules, edible coatings, or films. The results from this study are useful to prevent the damage caused by *R. stolonifer* in fruit products and also offer an eco-friendly alternative to reduce the use of synthetic fungicides and decrease jackfruit postharvest losses.

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Table 3. Chemical compounds identified by HPLC-UV-Vis in the D. carthagenensis extract.

Identified compound	Molecular formula	RT (min)	Extract concentration (mg/mL)	Antifungal activity*	Reference
Caffeic acid	$C_9H_8O_4$	1.95	1.73	-Inhibition of 1,6-*β*- and 1,3-*β*-glucan synthase -Fungal cell permeability	(Ma & Ma, 2015; Possamai-Rossatto *et al*., 2021)
Chlorogenic acid	$C_{16}H_{18}O_9$	1.33	1.23	-Membrane permeabilization in fungal spores -Reduction of spore germination -Decreased ability to form appressoria -Inhibition of 1,6-*β*-glucan synthase	(Ma *et al*., 2007; Martínez *et al*., 2017)
Gallic acid	$C_7H_6O_5$	0.74	10.03	-Inhibition of ergosterol biosynthesis (non-regulation of membrane-bound enzymes and membrane permeability)	(Li *et al*., 2017)
Vanillic acid	$C_8H_8O_4$	1.95	1.98	-Growth reduction -Alteration of cellular metabolism -Causes oxidative stress and hyphal injury	(Yousaf *et al*., 2023)
Scopoletin	$C_{10}H_8O_4$	2.55	1.64	-Fungal cell permeability -Increases the nucleotide release -It can bind to ABC-like transport proteins to hinder tertiary structure and inhibit the protein functions	(Vilchis-Gómez *et al*., 2024)
Mangiferin	$C_{19}H_{18}O_{11}$	0.65	0.28	-Cell wall disruption -Protein and enzyme leakage -Damage to genetic material -Inhibition of protein synthesis	(Kumar *et al*., 2021)
Vanillin	$C_8H_8O_3$	2.35	2.70	-Destroys the integrity of cell membranes (inherent components, synthases, ion channels/ transporters) -Disrupts cell walls (inner and outer layers) -Produces oxidative bursts that attack membrane lipids	(Li *et al*., 2021; 2022)

RT: retention time, * Antifungal activity reported in other studies.

Source: Own elaboration based on results.

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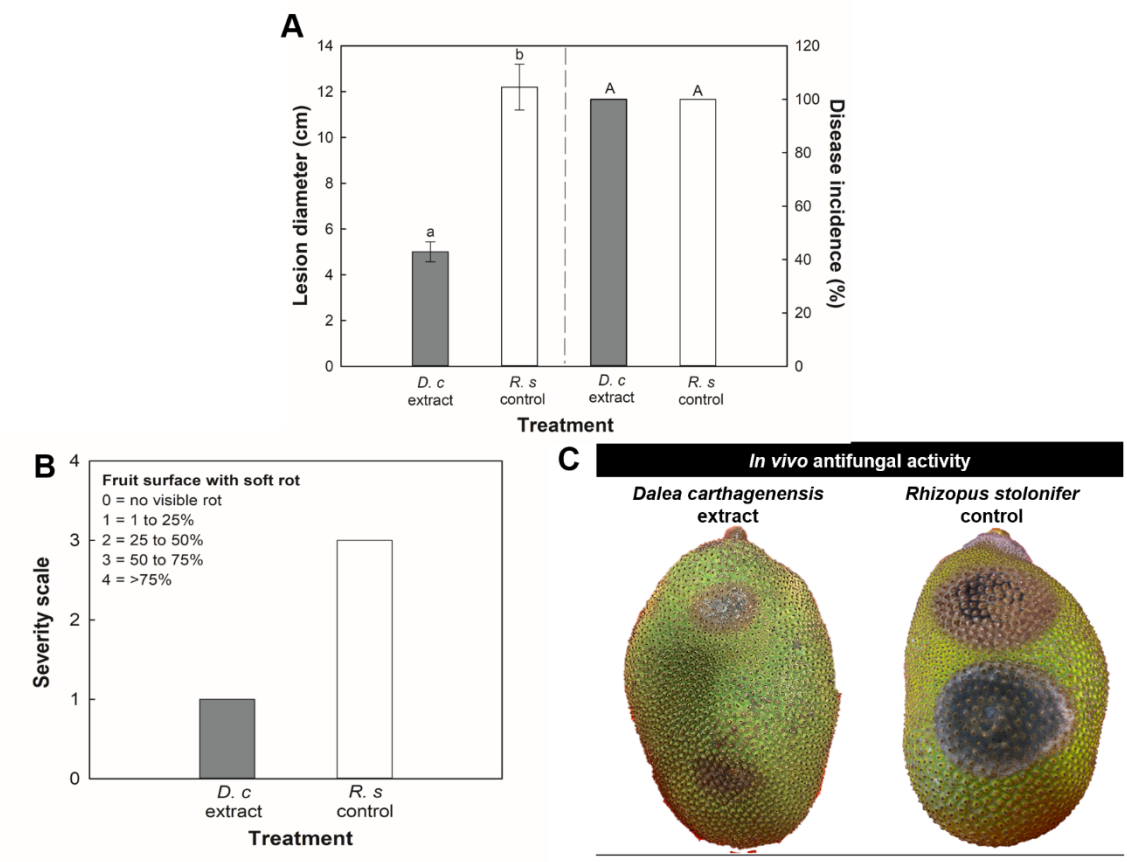

Figura 2. Inhibición *in vivo* de *R. stolonifer* con extracto de *D. carthagenensis* (A), escala de severidad de podredumbre blanda (B) y efecto del extracto en el control de la podredumbre blanda en el exocarpio de yacas almacenadas a 25 °C durante 5 días (C). *D.c*. extract: *D. carthagenensis* extract, and *R.s.* control: *R. stolonifer* control. Data are the means and the vertical lines represent the standard deviation. The experiment consisted of three repetitions with three replicates per treatment and five jackfruits per replicate. The mean values with the same lowercase letter and the values with the same capital letter indicate that there is no significant difference between the treatments according to the Fisher LSD test ($p < 0.05$). Source: Own elaboration based on results.

Conclusions

The efficacy and prospective use of *D. carthagenensis* extract was evidenced. The *in vitro* assay demonstrated the spectrum antifungal activity of *D. carthagenensis* extract against *R. stolonifer*. The *in vivo* antifungal activity of the extract suggests its potential use as an effective, safe, and ecological alternative to control the soft rot in jackfruits. The findings may contribute

to reducing the use of synthetic fungicides, decreasing postharvest losses, and offering a new natural control solution for jackfruit growers. In the future, the microencapsulation of the extract will be carried out to guarantee high bioactivity and stability of the HVBCs and increase its potential for future applications.

Authors contribution

Conceptualization (MCS, JARS), Investigation (MCS, KNGG, DSVG), Methodology (MCS, KNGG, DSVG, JEDR, JARS) Supervision (MCS, JARS), Validation (JARS), Funding acquisition (MCS, JARS), Writing - review & editing (MCS, KNGG, DSVG, JARS), Visualization (MCS), Data curation (KNGG), Formal analysis (KNGG, DSVG, JEDR), Project administration (JARS). All authors of this manuscript have read and accepted the published version

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Conflict of interest

The authors declare that they have no conflict of interest.

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