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*Phytopathological Note*

# *In vitro* antifungal activity and phytochemical characterization of aqueous extracts from *Datura discolor*

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## ABSTRACT

**Background/Objective.** *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* are phytopathogenic fungi of agricultural significance. The use of phytoextracts with antifungal properties offers an alternative approach to reduce agrochemical applications in pathogen management. This study reports the phytochemical characterization of *Datura discolor* aqueous extracts obtained by infusion and High-Pressure Processing (HPP), as well as their antifungal evaluation.

**Materials and Methods.** Aqueous extracts from the root, stem, seed, and leaf of *Datura discolor* (2, 4, and 6 % w/v) were obtained by infusion HPP. Phytochemical analysis was conducted through screening tests and quantification of total metabolites using colorimetric assays. The antifungal activity of the extracts obtained by infusion was determined based on the *in vitro* inhibition percentage of the pathogens.

**Results.** Total phenolics and saponins content in root, stem, and leaf was higher in extracts obtained by HPP, whereas infusion showed greater values in the seed. Flavonoids were observed only in leaf extracts obtained by HPP. Alkaloid content was similar both infusion and HPP extracts. Phenols, saponins, flavonoids, alkaloids, tannins, terpenoids, coumarins, and betacyanins were detected, while anthraquinones and anthocyanins were not. The extracts inhibited *Sclerotium rolfsii* by 2 to 46 % but showed no effect on *Sclerotinia sclerotiorum.*

**Conclusion.** The results indicate that phenolic compounds and flavonoids contribute to the antifungal activity against the evaluated phytopathogens; the involvement of other non-analyzed compounds cannot be ruled out. Further studies under greenhouse conditions are required, applying the extracts either as foliar spray or soil treatment.

**Keywords:** Thorn apple, Plant Extract, Metabolites, Fungi

# INTRODUCTION

*Sclerotium rolfsii* and *Sclerotinia sclerotiorum* cause root and stem rot. Both polyphagous phytopathogens are associated with significant losses in crops of economic importance. *S. rolfsii* is predominantly found in tropical and subtropical regions (Gholami *et al*., 2019), whereas *S. sclerotiorum* presents a broader geographic distribution, affecting crops in temperate and subtropical areas (Ordóñez-Valencia *et al*., 2018). The diseases produced by fungi that affect agricultural crop yields are frequent. There is a growing concern over the resistance of the strains to the fungicides used for the chemical control of fungi, so new products are being sought for the management of these phytopathogenic agents. The use of plant extracts as antifungal agents can be an alternative; different *Datura* species have shown an inhibiting effect against diverse phytopathogenic fungi, although the effect varies according to the concentration and the part of the plant from which the extract is obtained, as well as the types of solvents used for the process (Öz, 2017).

*D. discolor* extracts have been evaluated against different phytopathogens; methanolic and ethanolic leaf and stem extracts inhibited the *in vitro* development of *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium expansum*, *Fusarium moniliforme* and *Fusarium poae* (Tequida-Meneses *et al*., 2002). In addition, the aqueous leaf extract obtained by infusion affected the growth of *Colletotrichum gloeosporioides* (Verdugo-Contreras *et al*., 2023). Likewise, aqueous extracts (2, 4 and 6 % p/v) from roots, seeds and leaves obtained with high-pressure processing (HPP) displayed variable percentages of *in vitro* inhibition against *S. rolfsii*, *S. sclerotiorum* and *C. gloeosporioides*, with the 6 % leaf extract being the one that displayed the highest effectiveness against the three fungi (Urias-Lugo *et al*., 2024). The effect of *D. discolor* aqueous extracts obtained by infusion against *S. rolfsii* and *S. sclerotiorum* has not been evaluated.

The biological activity of the species of the *Datura* genus has been attributed to the presence of secondary metabolites such as phenolic compounds, flavonoids and alkaloids, which perform antioxidant and antimicrobial activities (Céspedes-Méndez *et al*., 2021). Despite *D. discolor* being widely distributed in Mexico, reports on the phytochemical characterization of this species are scarce (Verdugo-Contreras *et al*., 2023). Withanolides have been reported (González *et al*., 2023), as well as the presence of diverse groups of metabolites in raw leaf extracts, including alkaloids, steroids, terpenoids, glucosides, saponins, phenolic compounds and lipids (Ahanotu *et al*., 2024), which highlights the bioactive and biotechnological potential of this little-studied species.

The aim of this study was to identify the main groups of phytochemical compounds found in the aqueous *D. discolor* extracts obtained by infusion and HPP, and to evaluate the *in vitro* antifungal potential of the aqueous *D. discolor* extracts obtained by infusion against *S. rolfsii* and *S. sclerotiorum*.

**Obtaining the extracts.** The *D. discolor* plants were gathered in the flowering stage and fruition in April 2024 in the municipality of Ahome, Sinaloa, Mexico (25°54–55' N and 108–109°02–55' W). Disinfection and pulverization were carried out following the descriptions by Urias-Lugo *et al*. (2024). The aqueous extracts were prepared at a ratio of 1:10 p/v. For the extraction by infusion, the procedure by Bitwell *et al*. (2023) was followed, and for the extraction by HPP, the one by Urias-Lugo *et al.* (2024). For both

the infusion and the HPP methods, the mixtures were centrifuged (RC-5C Sorvall, Newtown, CT, USA) at 4000 rpm for 10 min, and the supernatants were extracted and stored at 4 °C until use.

**Phytochemical sieving of *Datura discolor*.** The main groups of bioactive compounds found in *D. discolor* aqueous extracts were identified with qualitative tests, as per reports by Khan *et al*. (2019). Color change tests were used for phenols, flavonoids, coumarins, tannins, terpenoids, anthraquinones, anthocyanins and betacyanins; precipitation was used for alkaloids and foam formation for saponins. Characterization was carried out using colorimetric and precipitation tests, specific to each type of compound, and the results were expressed on a semi-quantitative scale of relative concentration, ranging from absence (–) to abundant presence (++++).

For the detection of total phenols, 1 % ferric chloride was added, and its reaction with the extract produced green, violet, blue or black colors as a positive indicator. The presence of flavonoids was confirmed with the addition of concentrated sulfuric acid, with a characteristic orange color observed. Coumarins were identified with the formation of a yellow color when the extract was mixed with 10 % sodium hydroxide. The test for persistent foam formation lasting 15 to 20 minutes helped detect saponins. For tannins, adding 5 % ferric chloride after heating the extract resulted in green or blue colors, which indicated the presence of condensed or hydrolysable tannins, respectively. Terpenoids were identified with the Salkowski test, showing a reddish-brown color after mixing the extract with chloroform and concentrated sulfuric acid. Anthraquinones were detected using the method by Bontrager, observing a pink color due to the acid treatment, extraction with chloroform and alkalinization. Anthocyanins and betacyanins were differentiated by the color change to blue or yellow, respectively, when bringing the extract to a boil with NaOH at 0.1 N. Finally, the alkaloids were detected with Wagner's reagent, where the appearance of turbidity or a reddish-brown or unconfirmed their presence.

**Quantification of phytochemical compounds.** For the quantification of total metabolites, the liquid or lyophilized extracts were used (VirTis 25EL, VirTis Co. U.S.A.).

**Total phenolics**. This was determined using the colorimetric method by Singleton *et al*. (1999). A total of 10 µL of diluted extract (1:20 v/v) were placed in a 96-well microplate, followed by the oxidation of phenols with 100 µL of Folin-Ciocalteu reagent (1:10 v/v in water). After 2 min, the reaction was neutralized with 90 µL of 10 % $Na_2CO_3$. Subsequently, the sample was incubated at 40 °C for 30 min in the absence of light and absorbance was measured at 765 nm using a microplate reader (Synergy HT, Winooski, VT, USA). Gallic acid was used as a standard (0-0.4 mg $mL^{-1}$), and the results were expressed as µg, equivalents of Gallic Acid per mL of extract (µg EAG $mL^{-1}$).

**Total flavonoids**. The flavonoid content was determined using the colorimetric method by Quettier *et al*. (2000). A total of 50 µL of diluted extract (1:10) were placed in a 96-well microplate and 100 µL of 1.5 % $AlCl_3$ were added. After incubating for 10 min at 25 °C, absorbance was measured at 403 nm using a microplate reader (Synergy HT,

Winooski, VT, USA). Quercetin was used as a standard (0–0.15 mg $mL^{-1}$) and results were expressed as µg quercetin equivalents per mL of extract (µg EQ $mL^{-1}$).

**Saponins**. Quantification was carried out using the colorimetric method by Hiai *et al*. (1976), based on the formation of chromophore groups with vanillin and sulfuric acid. Twenty milligrams of freeze-dried *D. discolor* extract were dissolved in 80 % methanol, centrifuged at 4000 rpm for 2 min and the supernatant was collected. In a 96-well microplate, 20 µL of the extract were placed along with 8 % vanillin, maintaining the microplate in an ice bath for 2 min. Next, 200 µL of (cold) 72 % sulfuric acid were added, and it was stirred for 3 min. The sample was incubated at 60 °C for 10 min, cooled for 5 min and the absorbance was recorded at 470 nm (Synergy HT, Winooski, VT, USA). Diosgenin was used as the standard (0–0.4 mg $mL^{-1}$), and the results were expressed as µg diosgenin equivalents per mL of extract (µg ED $mL^{-1}$).

**Total alkaloids**. This was determined using the colorimetric method by Shamsa *et al*. (2008), with modifications. Four hundred milligrams of freeze-dried aqueous *D. discolor* leaf extract were defatted with 5 mL of hexane, followed by sonication (30 min) and centrifugation (4500 rpm/2 min), discarding the supernatant. The residual hexane was removed under a stream of nitrogen and the residue was resuspended in 5 mL of methanol, subjected to sonication and centrifugation, and the supernatant was collected. The methanol was evaporated in a rotary evaporator at 38 °C and the residue was resuspended in 1 mL of HCl 2 N, washed twice with 5 mL of chloroform and the pH was adjusted to neutral using NaOH 1 N. Then, 5 mL of phosphate buffer (2 M, pH 4.7) and 5 mL of bromocresol green solution (69.8 mg of BCG in 3 mL of NaOH 2 N and dilute to 1000 mL) were added. The formed complex was sequentially extracted with 1, 2, 3 and 4 mL of chloroform, stirring and collecting the lower phase and adjusting the final volume to 10 mL with chloroform. Finally, absorbance was measured at 470 using a microplate reader (Synergy HT, Winooski, VT, USA). An atropine calibration curve was constructed, and results were expressed as µg atropine equivalents per mL of extract (µg EA $mL^{-1}$).

***In vitro* antifungal evaluation**. The identity and phytopathogenicity of *S. rolfsii* and *S. sclerotiorum* have been described earlier; these pathogens are a part of the collection of the Environment and Health Research Unit of the UAdeO (Martínez-Álvarez *et al*., 2021; Martínez-Ereva, 2022). The experiments were performed using infusion-derived extracts diluted to different concentrations (2, 4 and 6 %; v/v) in PDA (PDA; Bioxon, Cuautitlán Izcalli, Estado de México, México) in Petri dishes, following the methodology described by Urias-Lugo *et al*. (2024). The experiments were conducted twice in a completely randomized design with four replicates per treatment and Petri dish as the experiment unit.

**Statistical analysis**. All data were subject to a Shapiro-Wilk normality test. For the *in vitro* test against pathogens, a one-way ANOVA was performed and for the data of phytochemical compounds, a two-way ANOVA was performed, followed by a Tukey test (Little and Hills 1978) with a value of $P < 0.05$. To allow values of zero in some treatments, the data were transformed using $\sqrt{x+1}$ as described by Gómez and Gómez (1984). Additionally, Student's unpaired t test ($P < 0,05$) was used to compare the alkaloid

concentrations. The statistical analyses were carried out in the GraphPad Prism software, version 6.00 for Windows (GraphPad Software).

**Phytochemical characterization.** Phytochemical screening detected phenolic compounds, saponins, flavonoids, alkaloids, tannins, terpenoids, coumarins and betacyanins, but no anthraquinones or anthocyanins in any of the extracts (Table 1). The root and stem extracts presented a lower metabolite diversity and content. The seed and leaf extracts presented the same metabolite families, although their concentrations were different. The HPP extraction method displayed a higher phenol and saponin extraction/retention in leaves, and of alkaloids, saponins and tannins in the seed, whereas the infusion presented a higher content of flavonoids and coumarins in the seed. This analysis helped establish a preliminary phytochemical profile for *D. discolor*, with potential implications in its biological activity.

**Table 1.** Detection of phytochemical compounds from 6 % aqueous *Datura discolor* leaf extracts obtained by infusion (INF) and by high-pressure processing (HPP).

| Group | Extraction methods | Anatomical part of the plant | | | |
|---|---|---|---|---|---|
| | | Root | Stem | Seed | Leaf |
| Phenolic compounds | INF | - | + | +++ | +++ |
| | HPP | + | ++ | +++ | ++++ |
| Saponins | INF | ++ | ++ | + | ++ |
| | HPP | +++ | +++ | ++ | ++++ |
| Flavonoids | INF | + | + | ++ | +++ |
| | HPP | - | - | + | +++ |
| Alkaloids | INF | + | - | - | +++ |
| | HPP | ++ | - | ++ | +++ |
| Coumarins | INF | - | - | ++ | +++ |
| | HPP | - | - | + | +++ |
| Tannins | INF | - | - | ++ | +++ |
| | HPP | - | + | +++ | +++ |
| Terpenoids | INF | - | - | ++ | +++ |
| | HPP | - | - | ++ | +++ |
| Anthraquinones | INF | - | - | - | - |
| | HPP | - | - | - | - |
| Anthocyanins | INF | - | - | - | - |
| | HPP | - | - | - | - |
| Betacyanins | INF | + | - | - | - |
| | HPP | + | + | - | - |

Based on the relative abundance observed in the preliminary screening and on the earlier evidence of its bioactivity, the phenolic compounds, flavonoids (Matías *et al*., 2020), saponins (Barile *et al*., 2007) and alkaloids (Singh *et al*., 2007) were chosen for quantification, due to their well-known involvement in plant defense mechanisms and their inhibiting potential against fungal pathogens. In general, a difference in

concentrations is observed between the plant tissue types and the extraction method (Figure 1 A-D): the content of phenolic compounds and flavonoids is greater in the leaf extract obtained with HPP (1370 µgEAG $mL^{-1}$ and 21.4 µgEQ $mL^{-1}$) than by infusion (586 µgEAG $mL^{-1}$ and 0.0 µgEQ $mL^{-1}$) (Figure 1 A and C), but the content of saponins and alkaloids is equal in leaf extract, regardless of the extraction method (Figure 1 B and D).

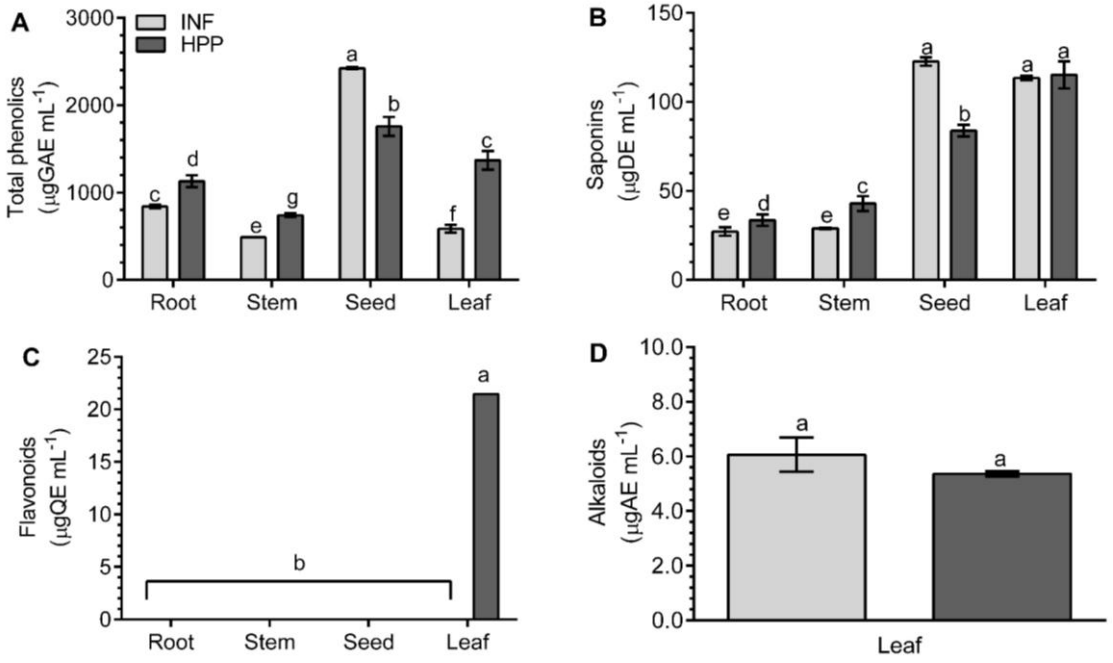


**Figure 1.** Quantification of phytochemical compounds from 6 % aqueous *Datura discolor* leaf extracts obtained by high-pressure processing (HPP) and infusion (INF). Gallic acid equivalents (EAG), Diosgenin equivalents (ED), Quercetin equivalents (EQ), Atropine equivalents (EA).

Phenolic compounds are directly related to the antifungal action, they can act as fungicides or fungistatic (Matías *et al*., 2020), they inhibit mycelial growth, in addition to acting on the emission of the germ tube of phytopathogenic fungi (Díaz-García *et al*., 2024). Meanwhile, the flavonoids act directly on cytoplasmic granulation, the disordering of the cell content, the rupture of the plasmatic membrane and the inhibition of enzymes produced by the fungi during the penetration of the host (Knaak and Fuiza, 2010). Phenolic compounds—both flavonoids and phenolic acids—have displayed antifungal activity against the phytopathogens *Alternaria alternata*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Botrytis cinerea* and *Phytophthora infestans* (Wianowska *et al*., 2016). Matías *et al*. (2020) related the antifungal potential of ethanolic and ethanolic *D. inoxia* leaf extracts against *S. sclerotiorum* with the abundance of alkaloids and phenolic compounds, mainly with total flavonoids.

**Antifungal evaluation.** The inhibition of the aqueous *D. discolor* extracts for *S. rolfsii* varied between 2 and 46 %. The highest inhibition was observed with the 6 % leaf extract (Table 2, Figure 2). Regarding the effect against *S. sclerotiorum*, the inhibition presented

by the leaf extract (4 and 8 %) was not significant (Table 2, Figure 2). In the *in vitro* evaluation, the stem extract was discarded, given the low percentage of extraction in relation to the one for leaf (10 *vs* 55 %).

**Table 2.** *In vitro* inhibition of the mycelial growth of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. The treatments shown are with aqueous *Datura discolor* extracts obtained by infusion.

| Treatment | | *Sclerotium rolfsii* | | *Sclerotinia sclerotiorum* | |
|---|---|---|---|---|---|
| | | **Growth (cm)** | **Inhibition (%)** | **Growth (cm)** | **Inhibition (%)** |
| **Root** | 2 % | 1.85±0.08 $^{ab}$ | 8 | 2.03±0.02$^{a}$ | 0 |
| | 4 % | 1.67±0.11 $^{b}$ | 17 | 2.08±0.03 $^{a}$ | 0 |
| | 6 % | 1.28±0.03 $^{c}$ | 37 | 2.03±0.07 $^{a}$ | 0 |
| | Control | 2.03±0.01 $^{a}$ | 0 | 2.02±0.02 $^{a}$ | 0 |
| **Seed** | 2 % | 1.97±0.09 $^{a}$ | 2 | 2.07±0.04 $^{a}$ | 0 |
| | 4 % | 1.48±0.15 $^{b}$ | 25 | 2.05±0.04 $^{a}$ | 0 |
| | 6 % | 1.26±0.13 $^{b}$ | 37 | 2.06±0.01 $^{a}$ | 0 |
| | Control | 2.03±0.01 $^{a}$ | 0 | 2.02±0.02 $^{a}$ | 0 |
| **Leaf** | 2 % | 1.35±0.02 $^{b}$ | 33 | 2.07±0.04 $^{a}$ | 0 |
| | 4 % | 1.26±0.01 $^{bc}$ | 38 | 1.94±0.07 $^{a}$ | 4 |
| | 6 % | 1.05±0.17 $^{c}$ | 46 | 1.85±0.01 $^{a}$ | 8 |
| | Control | 2.03±0.01 $^{a}$ | 0 | 2.02±0.02 $^{a}$ | 0 |

Means with common letters in superscript (by anatomical part of the plant) are not significantly different according to Tukey's test ($P \leq 0.05$; n = 8). Control = the pathogen in PDA with no extract.

Regarding the antifungal activity of *Datura* species against the pathogens used in this study, Jaben *et al.* (2014) and Jaben *et al.* (2022), reported a reduction in growth between 69 and 94 % and between 29 and 88 % of *S. rolfsii* respectively, with methanolic *D. metel* fruit and leaf extracts. Higher activity may be due to the species of *Datura* and to the extraction solvent used. On the other hand, the aqueous *D. discolor* leaf extract obtained by HPP inhibited up to 100 % of the *in vitro* mycelial growth of *S. rolfsii* (Urias-Lugo *et al.*, 2024), possibly due to the extraction process used.

Regarding *S. sclerotiorum*, Urias-Lugo *et al.* (2024) reported that the aqueous extracts obtained by HPP inhibited up to 56 % of this fungus and Matías *et al.* (2020) observed that the aqueous *D. inoxia* leaf extracts obtained by constant static maceration for seven days inhibited the growth of *S. sclerotiorum* by up to 94 %. The effectiveness observed against the pathogen by the aqueous extracts can be attributed to differences in the

extraction processes and in the type of bioactive compounds extracted in each one of the species.

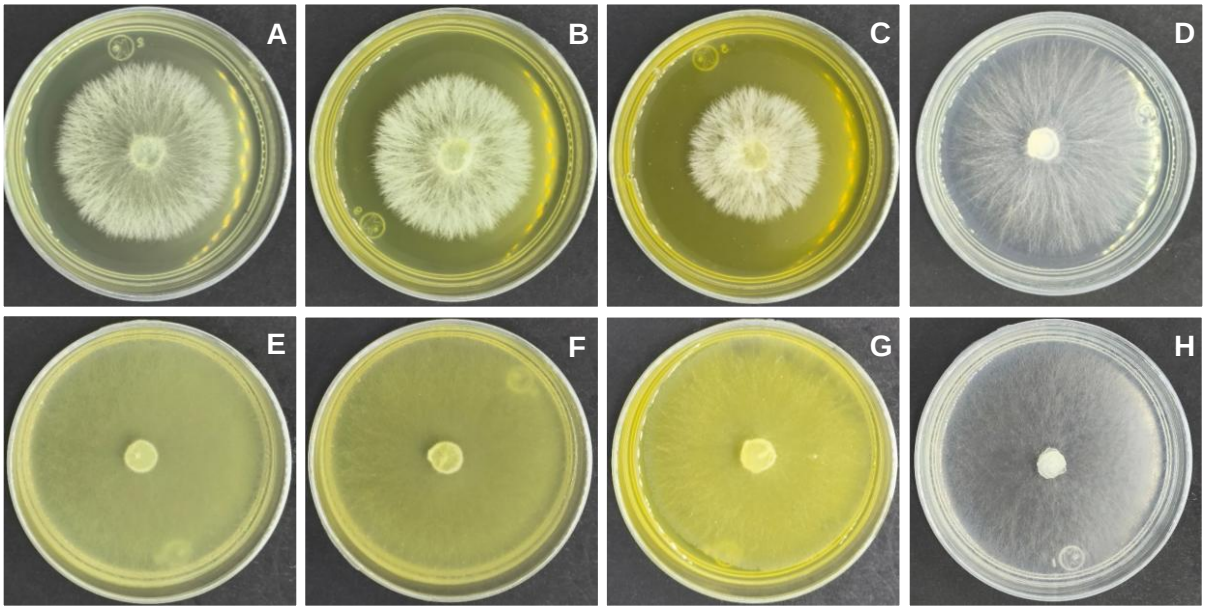


**Figure 2.** Effect of an aqueous *Datura discolor* leaf extract obtained by infusion. *Datura discolor* (2, 4 and 6 %) and Control, respectively. **A-D** *Sclerotium rolfsii*, **E-H** *Sclerotinia sclerotiorum.*

The phytochemical compound extraction processes that are based on thermal treatments can lead to the loss and degradation of thermolabile compounds with biological activity (Zhang *et al*., 2018), whereas HPP provides a high impact on the cell structure, destroying cell walls and other structural barriers, which enhances the recovery of bioactive compounts (Le-Tan *et al*., 2022). This may explain the effectiveness observed of the aqueous *D. discolor* extracts between the methods of infusion and HPP against *S. rolfsii* and *S. sclerotiorum*. The extraction processes used display differences in the phytochemical constituents of the aqueous *Datura discolor* extracts obtained by infusion and HPP, which exert a direct influence on the antimicrobial activity, since solubility, stability and synergy of the secondary metabolites extracted determine the degree of inhibition of the pathogens.

The results reported by Urias-Lugo *et al*. (2024), along with those from this study suggest the participation of flavonoids in the inhibition observed against *S. sclerotiourm* and *S. rolfsii* by the aqueous *D. discolor* leaf extracts obtained with HPP. In this study, the presence of flavonoids in the leaf extract obtained with HPP was only found with the quantitative method (Figure 1C). This could explain the lack of inhibition observed of the aqueous *D. discolor* extract obtained by infusion against *S. sclerotiorum*. Likewise, the extracts prepared by infusion also displayed inhibiting activity against *S. rolfsii*, but with a lower efficiency than the extracts obtained by HPP, indicating the possible presence of other bioactive compounds with antifungal effect, but with a lower potency or different action mechanism in comparison with the flavonoids extracted using HPP.

The results of this study do not help establish definitive conclusions regarding the secondary metabolites responsible for the antifungal activity observed, therefore the possibility of other compounds, not analyzed, found in the aqueous *D. discolor* extracts contributing individually or synergically to the inhibition of *S. rolfsii* and other evaluated fungi is not ruled out. This phytochemical complexity highlights the need for additional studies, aimed at the identification and characterization of the active compounds involved and their possible interactions.

Further studies should be conducted under greenhouse conditions, spraying leaves or the soil to observe the ability of control of the aqueous *D. discolor* extracts obtained by HPP and infusion, in order to confirm the reproducibility of the results observed under controlled conditions.

**Conflict of interest**

The authors declare no conflicts of interest.

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**Contributions of authors**

**Conceptualization**: DAUL, GAMR. **Experiment designs**: DAUL, GAMR**. Experiment executions**: DAUL, GLA, OEME, LACA**. Statistical analysis**: CRIS, LGSL**. Data interpretation**: DAUL, GLA, OEME, SPDC, GAMR. **Manuscript preparation**: DAUL, GLA, SPDC, GAMR. **Manuscript revision and approval**: all authors.

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