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Biological effectiveness of *Agave striata* and *Fouquieria splendens* extracts against *Pythium aphanidermatum* and *Rhizoctonia solani in vitro*

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ABSTRACT

Background/Objective. *Pythium aphanidermatum* and *Rhizoctonia solani* are pathogens that affect agricultural crops. The objective of this study was to evaluate some *Agave striata* and *Fouquieria splendens* methanolic extracts, from the Chihuahuan Desert, against those fungi, in search for biological control alternatives.

Materials and Methods. Pathogens from chili plants were isolated and identified using morphological and molecular methods. Methanolic extracts from both plants were prepared, and the antioxidant capacity (AC), the total polyphenol content (TPC), and the antifungal compounds via HPLC-MS were assessed. The antifungal effectiveness was tested at concentrations of 3.9-2000 mg L^{-1} using a poisoned medium assay, where fungi structural damage was observed.

Results. The *F. splendens* and *A. striata* extracts exhibited 55% and 68% AC, as well as 61 mg/g and 112 mg/g TPC, respectively. Both extracts contained caffeic acid and quercetin, while *F. splendens* also exhibited eriodyctiol, kaempferol, and luteolin; *A. striata* contained pinocembrin and theaflavin B. *F. splendens* attained 100% inhibition of *P. aphanidermatum* at 250 mg L^{-1}, and of *R. solani* at 500 mg L^{-1}, whereas *A. striata* achieved 100% inhibition at 1000 mg L^{-1} in both cases. The extracts produced lysis in *P. aphanidermatum* oogonia and mycelial fragmentation in *R. solani*.

Conclusion. The *F. splendens* and *A. striata* methanolic extracts demonstrate promising antifungal activity against *P. aphanidermatum* and *R. solani*, suggesting

that these natural compounds might be useful as a biological alternative for pathogen control in agricultural crops.

Keywords: plant extracts, biological effectiveness, phytochemicals, phytopathogenic fungi.

Introduction

Pythium aphanidermatum and *Rhizoctonia solani* are widely recognized pathogens due to their ability to cause devastating diseases in a variety of important agricultural crops. *P. aphanidermatum* causes rotting of the root, it affects the root system and causes the death of preemergent seedlings via aqueous lesions in the root and stem (Punja and Yip, 2003; Grijalba *et al.*, 2015). On the other hand, *R. solani* affects stems from the base, causing soft rots and root damages that weaken and kill plants prematurely (Medeiros *et al*., 2015). The persistence and adaptation of these phytopathogens in the soil complicate their management, affecting food security and the agricultural economy. Due to this, sustainable alternatives are currently being sought, such as plant extracts, with plants from the Chihuahuan Desert standing out as an option for the production of plant extracts due to their phytochemical composition and their ability to inhibit the development of phytopathogenic microorganisms (Tucuch-Pérez *et al*., 2021).

In this sense, *Fouquieria splendens* and *Agave striata* coexist and develop within the Chihuahan Desert. *A. striata* or "espadín" agave has a wide distribution in the south, from the Mexican state of Coahuila down to Hidalgo (Gentry, 1982), whereas *F. splendens* or "ocotillo" is distributed from southwestern United States to southern Zacatecas, Querétaro and Hidalgo (Henrickson, 1972). Investigations indicate that plants of the Chihuahuan Desert can inhibit or eliminate phytopathogenic microorganisms (Lira-Saldívar, 2003) that cause devastating diseases in economically important crops (Martínez *et al*., 2016; Hernández *et al*., 2018).

The control of phytopathogenic microorganisms is attributed to the environmental factors found in these regions, which stimulate the production of antimicrobial compounds in wild plants (Larios *et al*., 2020; Salas *et al*., 2023). The Agavaceae and Fouquieriaceae families have stood out for their antimicrobial properties (Gonzáles *et al*., 2015; Ramírez *et al*., 2023). *A. striata* and *F. splendens* are currently known to be used in the treatment of diverse illnesses in traditional medicine (Pérez *et al*., 2003; López *et al*., 2022), and have displayed antimicrobial properties against *Clavibacter michiganensis* subsp *michiganensis*, presenting phytochemicals such as alkaloids, carbohydrates, flavonoids, saponins, tannins and

quinones (Ramírez *et al*., 2023). The aims of this investigation were to identify phytochemical compounds found in methanolic *A. striata* and *F. splendens* extracts, to determine their Antioxidative Capacity (AC) and Total Polyphenol Content (TPC), to evaluate their biological effectiveness *in vitro* on *P. aphanidermatum* and *R. solani,* and to observe morphological changes in their structures due to the plant extracts.

Materials and Methods

Collecting plants. *A. striata* leaves and *F. splendens* stems were gathered in the municipal area of General Cepeda, Coahuila, Mexico (25°20'27.13'N and 101°27'52.07''W at 1531 m.a.s.l.), in the month of August. They were placed in black plastic bags and transported to the Mycology and Biotechnology Laboratory of the Agricultural Parasitology Department of the Universidad Autónoma Agraria Antonio Narro. The plant material was washed with tap water, then placed in a drying oven (Arsa AR-130D, Mexico) at 60 °C until its weight became constant. Finally, they were ground with a mill (Surtek Mogra1, Mexico) and sieved with a 0.2 mm sieve for storage in dark and properly labelled containers until use.

Preparation of the extracts. The extracts were prepared following the method reported by Jasso *et al*. (2015), with some modifications, using 96% methanol as a solvent; 42 g of the powder of each plant were deposited in 500 mL flasks adding 375 mL of the solvent, and they were placed in a stirring hotplate (Thermo Scientific Cimarec, USA) for 72 h at 60 °C. The solvent was then separated using rotary evaporation (IKA RV 10 digital V, USA) at 150 rpm at 70 °C. Once the methanolic fraction was obtained, it was poured into glass containers and placed in a drying oven (Arsa AR-130D, Mexico) at 50 °C for 72 h. Finally, the extracts were pulverized and stored in amber colored bottles in refrigeration at 4 °C.

Characterization of phytochemicals found in the *Agave striata* and *Fouquieria splendens* extracts with Reverse-phase liquid chromatography (HPLC-MS). The reverse-phase High-Performance Liquid Chromatography was carried out following the method by Ascacio *et al*. (2016), which consists of using an HPLC system. It varies in that it includes an automatic injector (Varian ProStar 410, USA), a three-way pump (Varian ProStar 2310, USA) and a PDA decanter (Varian ProStar 330, HPLC-MS). A mass spectrometer with a liquid chromatograph ion trap (Varian 500 – MS IT Mass Spectrometer, USA), equipped with a source of electrons by electrospraying was also used. Samples (5 µL) were injected into a Denali C18 column (150 mm x 2.1 mm, 3 µm, Grace, USA). The oven temperature

was maintained at 30 °C. The eluents were formic acid (0.2 %, V/V; solvent A) and acetonitrile (solvent B). The following gradient was applied: initial, 3% B; 0 – 5 min, 9% B linear; 5 – 15 min, 16% B linear; 15 – 45 min, 50% B linear. After the column was washed and reconditioned, the flow rate was maintained at 0.2 mL/min and the elution was monitored at 245, 280, 320 and 550 nm. All the effluent (0.2 mL/min) was injected into the mass spectrometer source, without splitting. All MS experiments were conducted in a negative mode [M-H]-1. Nitrogen was used as a nebulizer gas and helium, as the buffer gas. The ion source parameters were: spray voltage 5.0 kV and capillary voltage and temperature were 90.0 V and 350 °C, respectively. The data were gathered and processes using the MS Workstation Software (V 6.9). The samples were first analyzed in full-scan mode, acquired in the m/z range 50 – 2000. 6,951.6

Antioxidant capacity DPPH in *Agave striata* and *Fouquieria splendens* extracts. This was determined according to descriptions by Brand *et al*. (1995) with some modifications and analyzed by spectrophotometry. For this, 50 mg of dry material was weighed, which was added 1.0 mL of 80% methanol. Subsequently, the samples were sonicated for 20 min and centrifuged for 15 min at 14000 rpm. Once the extract was obtained, an aliquot of 10 µL was taken and placed in 96-well microplates and 290 µL of DPPH reagent prepared with ethanol at 100 µM was added. The sample was then allowed to react in the dark for 30 min. After this time, the absorbance was measured at 515 nm. The DDPH percentage of inhibition was determined using the natural absorbance of methanol at 80% as a control. The AC DPPH was determined using the following formula:

Inhibition of DPPH (%) = Absorbance of target /Absorbance of extract × 100

Total capacity of polyphenols in *Agave striata* and *Fouquieria splendens* extracts. The TPC was determined using the Folin-Ciocalteu method described by Singleton and Rossi (1965) with some modifications. In the analysis, a 10 µL alquot was taken from the methanol extract at 80%, followed by the addition of 790 µL of distilled water, 50 µL of Folin and 150 µL of sodium carbonate at 20%. Subsequently, the sample was left to rest for 1 h in the dark, after which absorbance was measured in a microplate reader at 765 nm. The TPC was determined using a standard curve with gallic acid and expressed as GAE mg/g (equivalent milligrams of gallic acid per gram).

Isolation and identification of *Pythium aphanidermatum* and *Rhizoctonia solani*. Roots and stems of serrano pepper (*Capsicum annuum*) plants, Var. Platino, were gathered in the stage of flowering, with symptoms of wilting, in the municipal area of Escuinapa, Sinaloa, Mexico, with a randomized sampling taking six plants

per sample, and five tissue samples were planted for every Petri dish, performing six repetitions. The samples were disinfected following the methodology by Booth (1977), they were washed, cut and submerged in 3% chlorine for 3 min, to then be rinsed and dried. They were planted in a PDA medium and incubated at 28 °C for seven days. Fungi were purified with the hyphal tip technique, a method that helps obtain axenic or contaminant-free fungi. Subsequently, the morphological identification was performed using the taxonomic keys by Díaz *et al*. (2011) for the characterization of *P. aphanidermatum,* and by Watanabe and Matsuda (1966) for *R. solani*. The molecular identification was carried out by the National Laboratory of Agriculrutal, Medical and Environmental Biotechnology (LANBAMA) at the Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), using the ITS1 and ITS4 primers, which amplify the ITS region between the 18S ribosomal subunit, enabling a specific identification of the fungal species.

Antifungal activity of *Agave striata* and *Fouquieria splendens* extracts on the mycelial growth of *Pythium aphanidermatum* and *Rhizoctonia solani.* The antifungal activity was determined by the poisoned medium method proposed by Jasso *et al*. (2011). *A. striata* and *F. splendens* extracts were used at concentrations of 3.9 to 2000 mg L^{-1}, in addition to a negative control, which consisted of only a PDA culture without extract; four repetitions were used for every concentration. Discs measuring 0.4 mm in diameter with active *P. aphanidermatum* and *R. solani* mycelia and seven days' growth were placed in the poisoned medium and incubated at 28 ± 2 °C. The evaluation was carried out measuring the radial growth of the fungi on a daily basis, and concluded when the control completely covered the Petri dish; the experiment was held twice. The percentage of inhibition was determined with the following formula:

$$\text{Percentage of Inhibition} = (DC-DT/DC) * 100.$$

where DC is the diameter of the control treatment and DT is the diameter of the different concentrations.

Effect of *Agave striata* and *Fouquieria splendens* extracts on the structures and morphology of *Pythium aphanidermatum* and *Rhizoctonia solani.* The methodology by Khaledi *et al*. (2015) was used, with modifications, to evaluate the modifications induced by the extracts in *P. aphanidermatum* oogonia and *R. solani* hyphae, using the results from the 50% inhibitory concentration (CI_{50}) of *A. striata* and *F. splendens*. In order to obtain *P. aphanidermatum* oogonia, the poisoned medium was used with the concentrations of 199.17 and 61.22 mg L^{-1} with the *A. striata* and *F. splendens* extracts, respectively, in an Agar V8 culture medium

(20 g agar, 3 g of CaCO3, 160 mL of Campbell's V8 vegetable juice and 840 mL of distilled water), while for the growth of the *R. solani* mycelia, the PDA culture medium in the poisoned medium was used with the extracts at concentrations of 211.18 and 92.59 mg L^{-1}; in both experiments, a negative control without any extract was used. After seven days of cultivation, the morphological changes were evaluated under an optic microscope with 40X and 100X lenses. Both experiments were held using six repetitions and were conducted twice.

Statistical analysis. A Probit analysis was conducted of the *in vitro* trials to determine the IC_{50} of each concentration using the SAS program, V9.0., and with the results, an analysis of variance was carried out with the inhibitory concentrations; Tukey tests ($p<0.05$) were also carried out.

Results and Discussion

Characterization of phytochemicals found in *Agave striata* and *Fouquieria splendens* extracts with Reverse-phase liquid chromatography (HPLC-MS). The phytochemicals identified in the *A. striata* and *F. splendens* extracts are shown in Table 1. In the case of *A. striata*, the presence of two elegitannins (pedunculagin and terflavin B) was observed, along with a hydroxycinnamic acid (caffeic acid), a methoxyflavonol (brassidin), a flavonol (quercetin), a flavonone (pinocepmbrin) and a phenolic terpene (rosmadial). Several bioactive compounds have been reported in *A. striata*, including flavanols (derived from quercetin) (Almaraz *et al*., 2013), triterpenoid and steroidal saponins, as well as alkaloids, carbohydrates, flavonoids (flavanones and chalcones), saponins (triterpenoid and steroidal), tannins (phenol), quinones (anthraquinones and benzoquinones) and coumarins (Ramírez *et al*., 2023). In the case of *F. splendens*, the presence of two flavonols (kaempferol and quercetin) was found, along with two flavones (roifolin and leutin), two methoxyflavonols (patuletin and dimethylquercetin), one flavanone (eriodictyol), one hydroxycinnamic acid (caffeic acid), one dihydroflavonol (dihydroquercetin), and another polyphenol (Phlorin). In this sense, Nevárez-Prado *et al*. (2021) mention that the most characteristic phytochemicals of the *Fouquieria* genus correspond to flavonoids (leucocyanidin, kaempferol and quercetin), p-coumaric, caffeic, ferulic and ellagic acids, as well as coumarin and scopoletin. On the other hand, Rodríguez (2010) found carbonyl groups, phenolic hydroxyls, sterols, methylsterols, coumarins, saponins, sesquiterpene lactones and flavonoids in *F. splendens*. In the stems and leaves, flavonoids such as kaempferol, ermanine and cetine have been found, along with flavones such as apigenin, acacetin, luteolin and chrysoeriol. Among these, apigenin, ermanine, rutin and quercitin-3-O-glucoside were the

Table 1. Phytochemical compounds identified in methanolic *Agave striata* and *Fouquieria splendens* extracts characterized by reverse-phase liquid chromatography (HPLC-MS).

Specie	Phytochemical	Retention Time (min)	Mass	CAS	Family
Agave striata	Caffeic acid 4-O-glucoside	5.554	341.1	166735-99-5	Hydroxycinnamic acids
	Pinocembrin	21.192	255.0	480-39-7	Flavanones
	Pedunculagin II	30.902	785.1	87687-52-3	Ellagitannins
	Terflavin B	35.351	784.9	103744-86-1	Ellagitannins
	Brasidine	40.341	623.1	17331-71-4	Methoxyflavonols
	Quercetin 3-O-glucuronide	43.630	477.2	22688-79-5	Flavonols
	Rosmadial	49.237	343.9	85514-31-4	Phenolic terpenes
Fouquieria splendens	Caffeic acid 4-O-glucoside	5.525	341.5	166735-99-5	Hydroxycinnamic acids
	Patuletin 3-O-glucosyl-(1->6)-[apiosyl(1->2)]-glucoside	34.539	787.1	101021-30-1	Methoxyflavonols
	Kaempferol 3-O-xylosyl-rutinoside	35.796	739.1	31921-42-3	Flavonols
	Kaempferol 3-O-glucosyl-rhamnosyl-glucoside	37.228	755.0	136449-09-7	Flavonols
	Kaempferol 3-O-rutinoside	37.924	593.1	17650-84-9	Flavonols
	3,7-dimethylquercetin	39.085	329.0	2068-02-2	Methoxyflavonols
	Dihydroquercetin	40.224	303.0	480-18-2	Dihydroflavonols
	Rhoifolin 4'-O-glucoside	40.506	739	31498-83-6	Flavones
	Luteolin 7-O-rutinoside	41.399	593	20633-84-5	Flavones
	Quercetin 3-O-glucuronide	43.62	480.2	22688-79-5	Flavonols
	Eriodictyol	43.803	287.0	552-58-9	Flavanones
	Phlorin	47.363	286.9	28217-60-9	Other polyphenols

main phenolic compounds in the leaves (Wollenweber, 1994; Monreal-García *et al*., 2019). Triterpenes, triterpenoids, steroidal saponins and iridoid compounds have also been discovered have also been discovered (Takhtajan, 2009), along tieh phytosterols and long- and short-chain alkanes in the flowers; and compounds such as Dammaran-triterpene, fouquierol and isofouquierol in the stem, as well as dammarendiol in the roots (Hegnauer, 1989; Waterman 1985). On the other hand, pyxinol and ocotillol have been isolated, the latter being a triterpenoid saponin with antimicrobial activities (Bi *et al*., 2017). López *et al*. (2022) identified the main phenols in *F. splendens* leaves such as gallic acid, ellagic acid and kaempferol-3-β-glucoside. Furthermore, Ramírez *et al*. (2023) reported that the methanolic extract from *F. splendens* stems contains alkaloids, carbohydrates, flavonoids (flavanones, flavones, flavonols and chalcones), reducing sugars, saponins (triterpenoids), tannins (gallic acid derivatied, catechol derivatives, and phenols), and quinones (anthraquinones and benzoquinones). Plants, within their metabolisms, produce compounds with antimicrobial activities that can control diseases caused by phytopathogenic microorganisms. The extraction of these compounds and their analysis allow for their application against various phytopathogens, offering an

alternative for the management of diseases in agriculture (Hernández-Lauzardo *et al*., 2007). In this sens, Ramírez *et al*. (2023) observed that *A. striata* and *F. splendens* extracts had antimicrobial activities against *Clavibacter michiganensis in vitro* and under greenhouse conditions, with the *F. splendens* extract being the most effective at reducing the incidence and severity of the disease. The *A. striata* and *F. splendens* extracts have displayed antifungal activity in further studies (Sánchez *et al*., 2005; Wang *et al*., 2010). However, their specific antifungal effect on *R. solani* and *P. aphanidermatum* has not been studied.

Antioxidant capacity DPPH in *Agave striata* and *Fouquieria splendens* extracts. The results showed that the *F. splendens* extract displayed a significantly greater antioxidant activity in comparison with the *A. striata* extract (Table 2). The inhibition of the DPPH radical was expressed as a percentage (%), being 68.7 for *F. splendens* and 55.9 for *A. striata*. In this sense, Garza *et al*. (2012) determined that the *F. splendens* methanolic extract had a mean effective concentration (EC_{50} considered as the sample concentration required to trap 50% of free DPPH radicals) of 130.2 µg. López *et al*. (2022) determined the antioxidant activity of *F. splendens* using DPPH results (2430.1 µmol TE/g), a Trolox Equivalent Antioxidant Capacity (TEAC) of 60.4 µmol TE/g, an Oxygenated Radical Absorbance Capacity (ORAC) of 4948.4 µmol TE/g and a Ferric Reducing Ability of Plasma (FRAP) of 7803 µmol Fe(II)/g) ($p < 0.0001$). In *Agave* there are investigations in the determinations of the antioxidant capacity. A study conducted by Ahumada *et al*. (2013) obtained results that displayed significant variations in the antioxidant activity between different species of *Agave*. In *Aagave rzedowskiana*, it presented the greatest antioxidant activity with 27.4 µM TE/g p.s., followed by *Agave ornithobroma* with 19.9 µM TE/g p.s.

Table 2. Antioxidant Capacity and Capacity of Total Polyphenols of methanolic *Agave striata* and *Fouquieria splendens* extracts.

Specie	AC Percentage of Inhibition	CTP (GAE mg/g)
Agave striata	55.90 ± 1.63[a]	61.2 ± 1.17[a]
Fouquieria splendens	68.71 ± 1.22[a]	112.2 ± 3.69[b]

*Values with the same letter are statistically similar (Tukey, $p<0.05$).

Capacity of total polyphenols in *Agave striata* and *Fouquieria splendens* extracts. The results showed that the *F. splendens* extract displayed significantly higher total polyphenols in comparison with the *A. striata* extract. The TPC was

expressed in CFT values CFT (GAE mg/g), being 112.2 for *F. splendens* and 61.2 for *A. striata* (Table 2). López *et al*. (2022) analyzed the phenolic compounds of the methanolic extract taken from *F. splendens* leaves, using the method by Folin Ciocalteu and after fractioning, they noticed that the ethyl acetate fraction contained the greatest number of phenolic compounds, with 479.9 mg GAE/g ($p < 0,0001$). In another study, Ahumada *et al*. (2013) found significant variations in the total phenol contents between species of *Agave*. *A. ornithobroma* had the highest value with 12.37 mg GAE/g p.s., while *Agave angustifolia* displayed the lowest value with 2.06 mg GAE/g p.s., indicating a considerable variability in the antioxidant and phenolic compounds between the species studied.

Isolation and identification of *Pythium aphanidermatum* and *Rhizoctonia solani*. Out of the Platino variety of serrano peppers with symptoms of wilting, *R. solani* and *P. aphanidermatum* were isolated and identified. In *R. solani* plae maroon hyphae were observed, 5-8 µm in width and branched in a 90 ° angle (Watanabe and Matsuda, 1966), whereas for *P. aphanidermatum*, coenocytic mycelium, sporangia and oogonia were observed (Díaz *et al*., 2011), which correspond to the species *R. solani* and *P. aphanidermatum*, respectively. Regarding molecular identification, the sequences provided by the LANBAMA of the IPICYT for each species were compared with the BLAST of the National Center for Biotechnology Information. In the case of *R. solani*, its sequence obtained a coverage of 100% and 100% similarity when compared with accession number JX535004.1. On the other hand, the sequence for *P. aphanidermatum* obtained a coverage of 100% and 100% similarity when compared with accession number EU245039.1. Finally, both sequences were deposited in the GenBank, obtaining accession numbers PQ571162 for *R. solani*, and PQ571194 for *P. aphanidermatum*.

Antifungal activity of *Agave striata* and *Fouquieria splendens* extracts on the mycelial growth of *Pythium aphanidermatum* and *Rhizoctonia solani*. The results of the plant extracts on the mycelial growth of *P. aphanidermatum* displayed a highly significant effect. *F. splendens* was the most effective, with an inhibition of 100% from 250 mg L^{-1}, whereas *A. striata* reached 100% inhibition at 1000 mg L^{-1} (Figure 1). The IC_{50} values were 61.2 mg L^{-1} for *Fouquieria splendens* and 199.2 mg L^{-1} for *Agave striata*, presenting statistical significance (Table 3). A study conducted by Milagrosa *et al*. (2007) reported that wine vinasse at concentrations of 5% inhibited *P. aphanidermathum* by 100%. Meanwhile, the results obtained from the plant extracts on the mycelial growth of *R. solani* displayed inhibition in the mycelial development of the pathogen. In turn, *F. splendens* presented an inhibition of 100% from 500 mg L^{-1}, and *A. striata* reached an inhibition of 100% starting at 1000 mg L^{-1} (Figure 2). The lowest IC_{50} was 92.6 mg L^{-1} for *F. splendens*

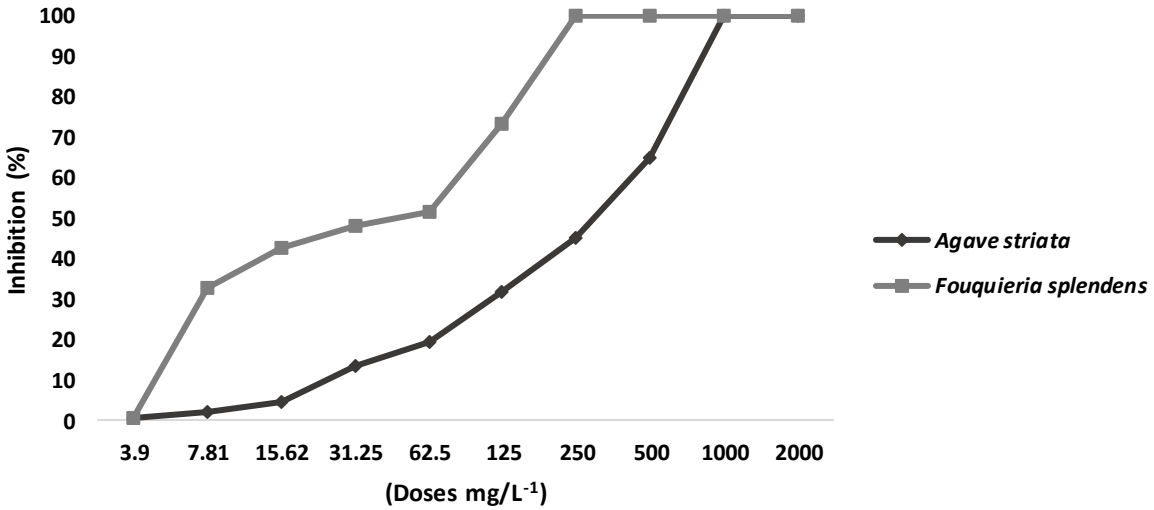

Figure 1. Inhibition of *Pythium aphanidermatum* with methanolic *Agave striata* and *Fouquieria splendens* extracts using the poisoned medium method.

Table 3. Inhibitory concentration at 50% (IC_{50}) of methanolic *Agave striata* and *Fouquieria splendens* extracts on *Pythium aphanidermatum* and *Rhizoctonia solani.*

	Pythium aphanidermatum	***Rhizoctonia solani***
Agave striata	199.17[b]	211.18[b]
Fouquieria splendens	61.22[a]	92.59[a]

*Values with the same letter are statistically similar (Tukey, $p<0.05$).

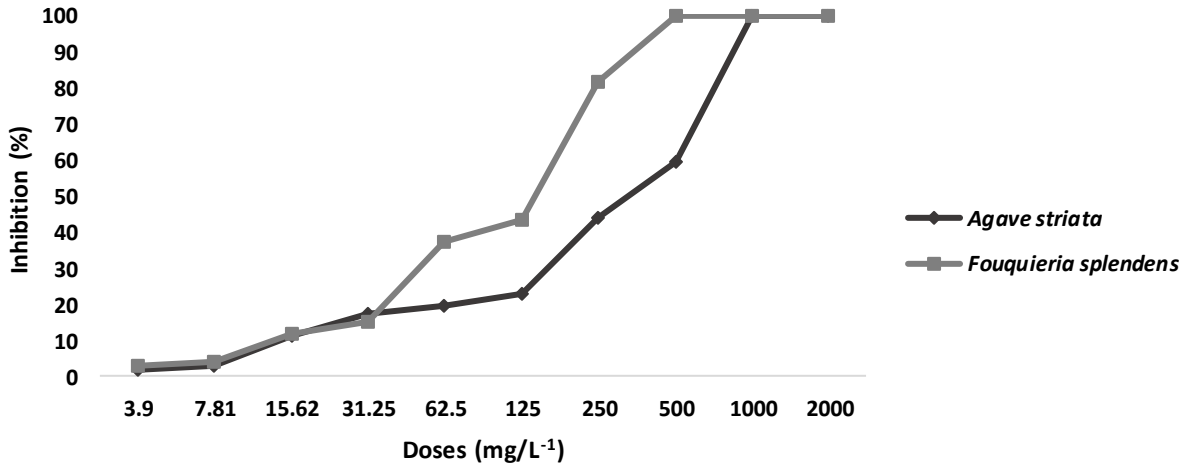

Figure 2. Inhibition of *Rhizoctonia solani* using plant *Agave striata* and *Fouquieria splendens* extracts using the poisoned medium method.

and 211.2 mg L^{-1} for *A. striata,* observing a statistical difference (Table 3). An investigation by Rodríguez *et al*. (2020) reported inhibitions of mycelial growth (IMG, %) in *R. solani* of 100 and 50% using *Larrea tridentata* and *Rosmarinus officinalis* extracts, respectively.

Caffeic acid, found in the *A. striata* and *F. splendens* extracts, has presented antifungal ability (Freires *et al.*, 2016), as observed in previous studies. An example of this is the study by Romero *et al*. (2009), who investigated the effect of phenolic compounds such as caffeic acid, rutin and quercetin in the growth of *Aspergillus carbonarius* and the production of ochratoxin A. They observed that all phenolic compounds, at a concentration of 250 mg L^{-1}, had a significant impact on the growth rate of *A. carbonarius*. In addition, Bisogno *et al*. (2007) reported that the minimum inhibiting concentration (MIC) of caffeic acid for *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus* was greater than 250 mg L^{-1}. On the other hand, the quercetin found in both extracts (*A. striata* and *F. splendens*) has displayed antifungal activity against five fungal species: *Alternaria alternata, Aspergillus fumigatus*, *A. niger*, *Macrophomina phaseolina* and *Penicillium citrii* (Kanwal *et al*., 2010). The flavonoid pinocembrin found in *A. striata* has anti-inflammatory, antimicrobial and antioxidant roles (Rasul *et al*., 2013) and has shown an inhibiting effect against *Candida albicans* (Hernández-Tasco *et al*., 2018; Tundis *et al*., 2018). The ellagitannin terflavin B, found in *A. striata*, has shown an inhibiting effect against *C. albicans* (Salih *et al*., 2022). In addition, the tannin pedunculagin, a phytochemical found in *A. striata*, exhibited an inhibiting effect against *A. flavus* in extractos *Punica granatum* extracts (Mostafa *et al*., 2011). Ilk *et al*. (2017) developed lecithin/chitosan nanoparticles loaded with kaempferol, in which they observed inhibitory activity against *Fusarium oxysporum*. However, pure kaempferol also proved to be effective against *C. albicans* (Yordanov *et al*., 2008). The flavonoids eriodyctiol, dihydroquercetin and luteolin, found in *F. splendens*, have displayed antifungal activity against *Fusarium graminearum* and *Septoria zeicola* in extracts of *Ficus sarmentosa* var. *henryi* after a fractionation process (Wang *et al*., 2010).

Effect of *Agave striata* and *Fouquieria splendens* extracts on the structures and morphology of *Pythium aphanidermatum* and *Rhizoctonia solani*. Both extracts displayed activity against *P. aphanidermatum* oogonia, leading to lysis at high doses of 2000 to 500 mgL^{-1} and malformation and deformation of hyphae at concentrations of 199.2 and 61.2 mg L^{-1} with the *A. striata* and *F. splendens* extracts (Figure 3). Lópeza *et al*. (2022) proved that *Bacillus amyleloquefaciens* completely inhibited the germination of *Phytophthora capsici* zoospores, causing encystment, malformations in the germinational tube and cell degradation. The use of plant extracts altered the morphology of the *R. solani* hyphae, which appeared distorted and fragmented with concentrations of 211.2 mg L^{-1} with the extract of

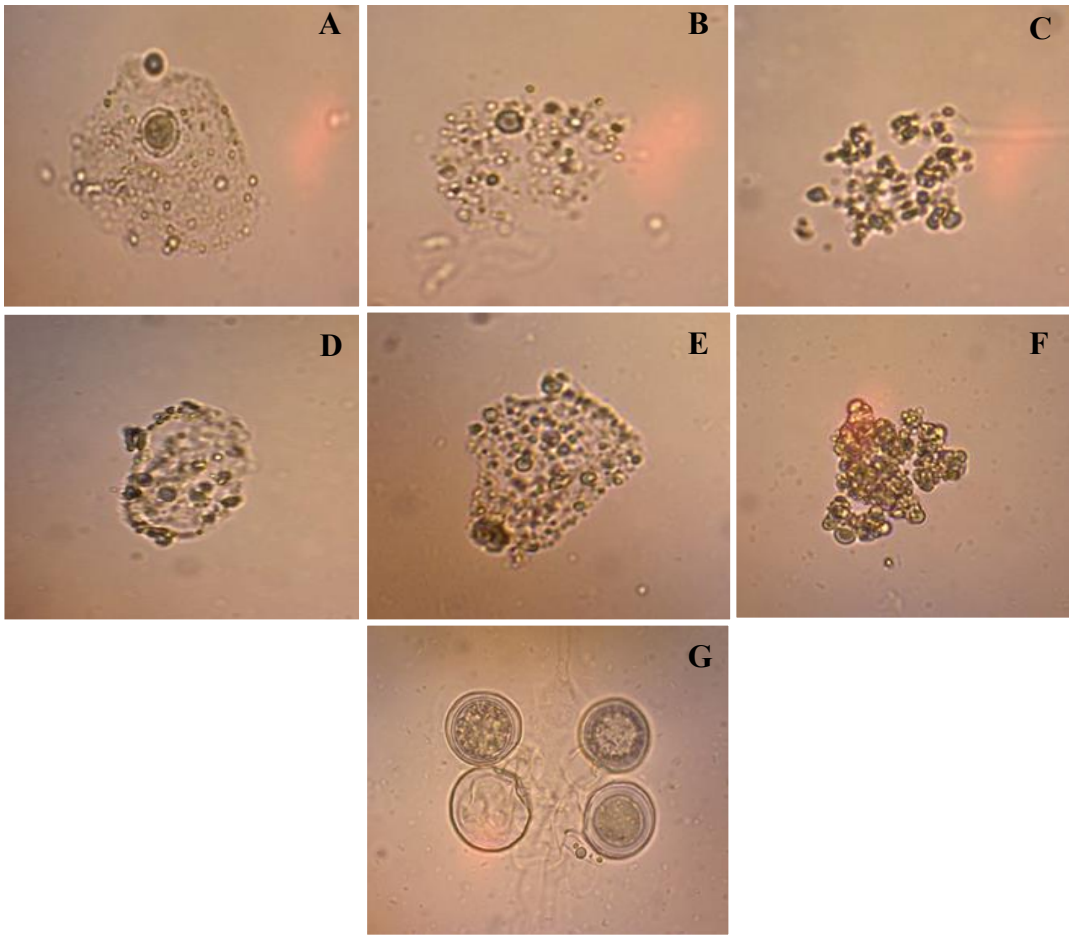

Figure 3. Effect of plant extracts on *Pythium aphanidermatum* oogonia at 100X. A, B and C) *Fouquieria splendens*, D, E and F) *Agave striata*, and G) Negative control.

A. striata and of 92.6 mg L^{-1} with the *F. splendens* extract (Figure 4), similar to descriptions by Khaledi *et al*. (2015) with *Mentha piperita*, *Bunium persicum* and *Thymus vulgaris* essential oils.

The results can be attributed to the presence of antifungal compounds in the *A. striata* and *F. splendens* extracts. For example, quercetin found in both extracts is a flavonoid that has been proven to reduce the content of ergosterol, a crucial sterol for the fluidity and stability of cell membranes, in *Trichophyton rubrum* (Bitencourt *et al*., 2013). It has also shown the inhibition of the enzyme CYP51, which is involved in the synthesis of steroids in fungi (Khanzada *et al*., 2021). The flavonoid pinocembrin, found in *A. striata*, has displayed different action mechanisms, including interference with energy homeostasis, increases in cell membrane permeability, reductions in the content of intracellular constituents (e.g.,

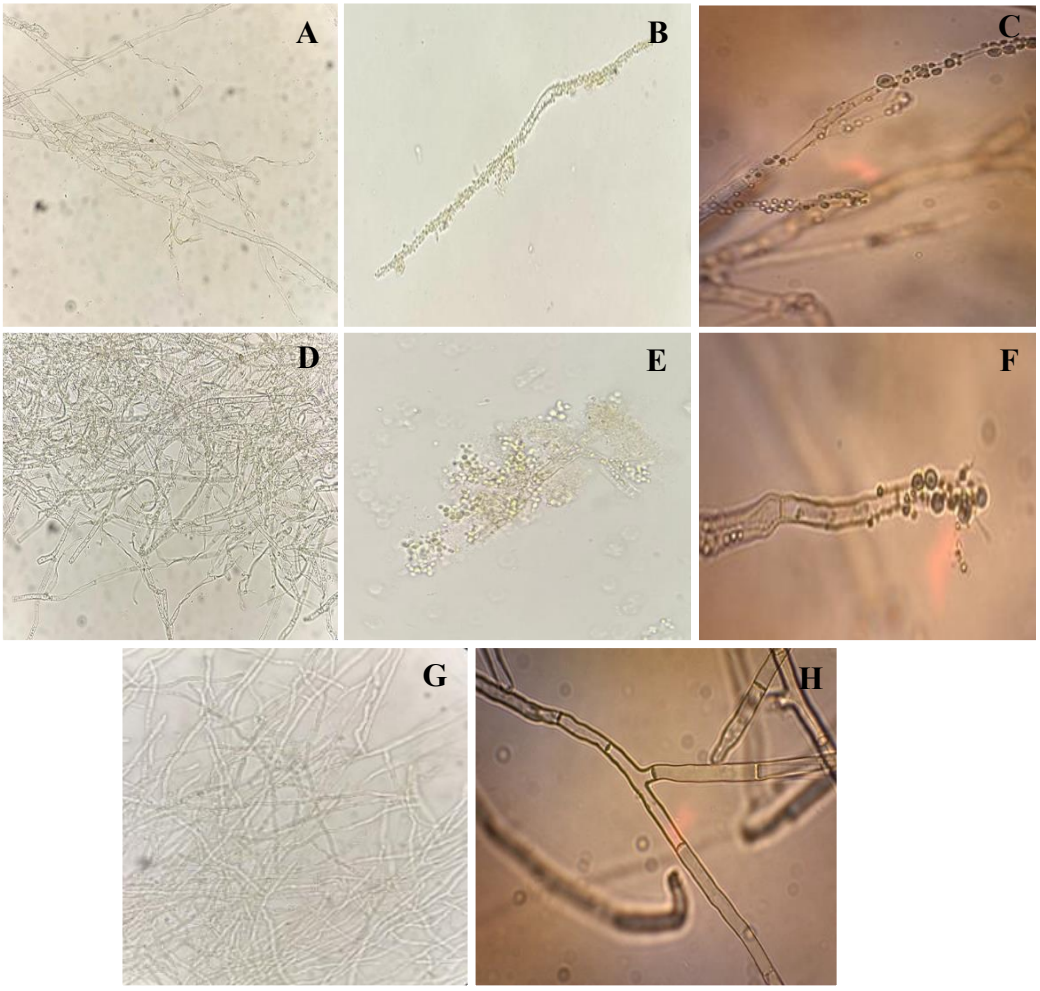

Figure 4. Effect of plant extracts on mycelial *Rhizoctonia solani* structures at 40X and 100X. A, B, C) *Fouquieria splendens*, D, E, F) *Agave striata*, and G), H) Negative control.

soluble proteins, reducing sugars and total lipids). Additionally, a marked reduction in chitin and glucanase contents was induced in the mycelia of *Penicillium italicum* (Peng *et al*., 2012; Chen *et al*., 2020). On the other hand, the flavonoid kaempferol found in *F. splendens* has been suggested in studies to cause damage to hyphae and is likely associated with cell wall alteration, leading to cell leakage and loss of cell integrity (Ilk *et al*., 2017). It has also shown synergistic antifungal activities with histone deacetylase (Rajasekharan *et al*., 2014), as well as the inhibition of the enzymes CYP51 and NDK, the latter being an enzyme involved in the phosphorylation of nucleosides and nucleotides (Khanzada *et al*., 2021).

Conclusions

Phytochemicals with antifungal potential were identified in *A. striata* and *F. splendens*. Both extracts contained caffeic acid and quercetin. Additionally, *A. striata* displayed the presence of pinocembrin and terflavin B, whereas *F. splendens* presented eriodyctiol, kaempferol, dihydroquercetin, luteolin and pedunculagin. On the other hand, *F. splendens* was found to display a significantly higher antioxidant activity and CFT than *A. striata*. Both extracts displayed biological efficiency against *P. aphanidermatum* and *R. solani*. Likewise, they also presented alterations in the morphology of *P. aphanidermatum* oogina and the *R. solani* hyphae. These results indicate that *A. striata* and *F. splendens* extracts have promising antifungal compounds, suggesting its potential in future phytopathogen control studies.

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