Full Length Article

High-Performance Thin Layer Chromatography Fingerprint Profile and Antifungal Activity of Methanolic Extracts of *Jatropha platyphylla*

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Abstract

Fungi of the genera *Colletotrichum* and *Fusarium* affect agricultural foodstuffs in crop yield and economic loss and the excessive use of antifungal treatments has resulted in antifungal drug resistance. Plants are rich sources of a wide variety of bioactive secondary metabolites reported to have *in vitro* antifungal properties. Therefore, this study was designed to perform phytochemical screening, high-performance thin layer chromatography (HPTLC) fingerprint profile, and *in vitro* antifungal activity of *Jatropha platyphylla* bark and leaf methanolic extracts against *Colletotrichum cliviae, Fusarium oxysporum* f. spp. *lycopersici* and *F. oxysporum* f. spp. *radicis-lycopersici*. The HPTLC chromatogram analysis confirmed the presence of terpenoids and flavonoids. Additionally, apigenin 7-*O*-glucoside was identified in bark and leaf samples and confirmed by the chromatogram standards by comparing its retention factor (Rf = 0.977). The methanolic bark and leaf extract added at a concentration of 0.05% stopped *C. cliviae* mycelium growth significantly by 96.50% and to a lesser degree to both *Fusarium* species. In light of the *in vitro test* results obtained, the methanolic extracts of *J. platyphylla* appeared to be an excellent source of antifungal components whose potential would allow the development of this species into treatments for the biological control of fungal diseases. © 2023 Friends Science Publishers

Keywords: HPTLC; *Jatropha platyphylla*; Mycelial inhibition; Phytopathogenic fungi; Secondary metabolites

Introduction

Phytopathogenic fungal infections are considered one of the major problems in agricultural industry due to their difficult control, affecting producers around the world with an annual loss of billions of dollars by damaging crops in the field and causing postharvest losses (Chen *et al.* 2018; Khan *et al.* 2020). Amongst the broad variety of fungal diseases, anthracnose – caused by *Colletotrichum* spp. – is one of the most harmful, limiting the global production of several crop varieties, such as chili, citrus fruits, rose apple, avocado, grapes, mango, and papaya. Preharvest anthracnose reduces yield while postharvest affects fruit quality, negatively impacting fruit export and marketability (Worku and Sahe 2018). Similar to anthracnose, Fusarium wilt, caused by *Fusarium oxysporum* f. spp. *lycopersici*, is another notorious fungal disease that affects tomato production by attacking the plant roots, causing heavy economic losses on plant growth (Smaoui *et al.* 2022). Furthermore, *Fusarium* species can produce mycotoxins in several types of cereals, fruits, and vegetables representing a major threat to human

and animal health since they are responsible for different types of toxicities (Price *et al.* 2015).

To combat these pathogens, farmers often depend on the rapid action and efficacy of synthetic fungicides. The widely available commercial fungicides used to control the plant fungal pathogens in the field are azoles, phenylamides, quinone-outside inhibitors (QoIs), dicarboximides, anilinopyrimidines and carboxylic acid amides (Vielba-Fernández *et al.* 2020). Although these fungicides have different modes of action and target sites, the resistance of numerous fungal pathogens to these fungicides has increased due to the constant use over the years making their treatment difficult (Casida and Durkin 2017). Moreover, most of these chemical residues have been well documented to be highly toxic for humans and organisms in the environment where they are sprayed because they can persist in the soil for years altering the ecosystem. Hence a great demand exists for safer, alternative, and effective antifungal agents (Matrose *et al.* 2021).

Among the strategies in controlling plant diseases, the use of natural compounds from medicinal and aromatic

plants offers a promising treatment to reduce the incidence of plant diseases (Broda 2020; Javaid *et al.* 2020; Jabeen *et al.* 2021). Many studies have revealed the potential of natural compounds, such as monoterpenes, phenolic compounds, flavonoids, alkaloids, or saponins, as bio-based herbicides, fungicides and insecticides (Bueso *et al.* 2016; Ferdosi *et al.* 2021; Javed *et al.* 2021).

Species from the genus *Jatropha* have been widely used in traditional folk medicine to cure various ailments (J Martins *et al.* 2021; amaluddin *et al*. 2022). Thus, several studies have focused on identifying compounds, including phenolics, terpenoids and alkaloids (Rahu *et al*. 2021; Sama *et al.* 2021). *Jatropha platyphylla* is a wild non-toxic species, endemic to Mexico (Ambriz-Pérez *et al.* 2017), which is known to have leaves and fruits that are rich in polyphenols and lipophilic compounds with anti-inflammatory activity (Leyva-Acuña *et al.* 2020). Recently, this research group has proven that the alkaloids obtained from *J. platyphylla* leaf methanolic extract inhibited *Aspergillus niger* growth (Dave *et al.* 2021). Based on findings of the previous results, this study tested the antifungal capabilities of methanolic extract from *J. platyphylla* bark and leaves *in vitro* against *F. oxysporum* f. spp. *lycopersici*, *F, oxysporum* f. spp. *radicis-licopersici* and *C. cliviae* to develop natural fungicides. All extracts were also subjected to phytochemical testing and high-performance thin layer liquid chromatography fingerprinting.

Materials and Methods

Chemicals, reagents and materials

The highest quality available reagents were purchased from Sigma-Aldrich®, USA and used without further purification. The reference substances used for the analyses were naringin, (95% purity), chlorogenic acid (95% purity), apigenin 7-*O*-glucoside (97% purity) and diosgenin (93% purity) (Sigma-Aldrich®, USA). For the HPTLC analysis, methanol, hexane, and ethyl acetate were HPLC grade (J.T. Baker®, USA), and purified water was used (PURELAB® Classic UV, ELGA LabWater, USA). The derivatizing agents used were 2-aminoethyl diphenylborinate at 98% and 400 polyethylene glycol (Sigma-Aldrich®, USA). Other chemicals and solvents were of analytical grade.

Plant material

Bark and leaves of *J. platyphylla* were obtained from specimens located in Ejido de la Campana (24° 53´ 52.3´´ N; 107° 27´ 18.3´´ W and 94 m a.s.l.) in Culiacán, Sinaloa, Mexico. The samples were transferred to the Bioresources Laboratory at CIAD (Centro de Investigación en Alimentación y Desarrollo), dried under shade at room temperature for one week and ground with an electric homogenizer (Oster®, USA) to obtain flour, of which 40 g were weighed and macerated with 400 mL of analytical degree methanol (FAGALAB, Mexico) with an orbital agitator S-500 (VWR International, USA) in darkness at room temperature for 24 h. The sample was vacuum filtered with a porcelain funnel and filter paper Whatman No.1 (Sigma-Aldrich®, USA). The filtering obtained was evaporated to dryness in a rotavapor (BUCHI, Canada) at 365 mbar, 45ºC and at 50 rpm until a total concentrate was obtained and stored at 4°C until use.

Phytochemical screening

Phytochemical screening was performed following the methodology reported by (Dave *et al.* 2021). Tannins were determined with the $FeCl_3$ test. The Shinoda test was used to determine phenolic compounds, the Dragendorff, Mayer and Wagner for alkaloids, foam for saponins and the Salkowski and Libermann for determining terpenoids.

High-performance thin layer liquid chromatography analysis

The studies performed were based on Reich's methodology (Reich *et al.* 2006) in a system of HPTLC (CAMAG, Switzerland) equipped with Linomat V applicator, TLC scanner and Visioncats software; 10 mg of the concentrated extracts were dissolved in 3 mL of MeOH, filtered using 0.45-µm nylon acrodiscs (MILLEX, Germany) in a HPTLC run, 4 μL of each sample. Standards (naringin, chlorogenic acid, apigenin 7-*O*-glucoside and diosgenine) were applied in band form in positions X and Y at 15 mm, 11 mm in length, and a track distance of 13.4 mm on a silica gel G-25 UV254 glass plate (10 cm × 10 cm) (MACHEREY-NAGEL, Germany) developed in a HPTLC (CAMAG, Switzerland) camera and saturated with a mobile phase of ethyl acetate: formic acid: and water (15:1:1) for flavonoids and hexane: ethyl acetate (6:4) for terpenoids at an optimum temperature of 25°C for 30 min. After that, the plate was left to develop in the mobile phase until a solvent front of 85 mm was reached.

Once the solvent front was reached, the plate was left to dry with cold air for five min, scanned with a densitometer (CAMAG, Switzerland) at 254 and 366 nm, and placed on a heating plate at 100°C for three minutes. The HPTLC plate-still hot was sprinkled with 2-aminoethyl diphenylborinate at 98% and 400 polyethylene glycol to reveal flavonoids and anisaldehyde in sulfuric acid for terpenoids (Orsini *et al.* 2019). Then, the plate was placed in an ultraviolet (UV) light visualizer (CAMAG, Switzerland) of 254 and 366 nm, and photographic images were taken. Finally, it was scanned again in densitometer at 366 nm for the chromatogram (Ramaiah and Garampalli 2015; Mishra *et al.* 2020).

Fungal material

The fungal strains *F. oxysporum* f. spp. *lycopersici* and *F.

oxysporum f. spp. *radicis-licopersici* donated by CIAD Plant Pathology Laboratory were isolated and identified from tomato crops from Culiacan Sinaloa, México. *Colletotrichum cliviae* was isolated and identified from papaya from Cotaxtla, Veracruz, México.

Antifungal activity

Antifungal activity of *J. platyphylla* bark and leaf methanolic extracts were tested in 50 mm Petri boxes. A total of 200, 100, and 50 mg of the extract was added at 100 mL of the PDA (potato dextrose agar) medium previous to gelification (approximately at 50°C), corresponding to concentrations of 0.2, 0.1 and 0.05%. Sterile water was added to the PDA medium, which served as the control. With the help of a puncher, 5 mm of the inoculated medium with *F. oxysporum* f. spp. *lycopersici*, *F. oxysporum* f. spp. *Radicis-licopersici* and *C. cliviae* were taken and placed face down in the center of the Petri dishes (60 mm) with the media prepared (Jug *et al.* 2018). The plates were inoculated at 25°C and waited until the control group was full to measure mycelium inhibition. The assay was performed in triplicate with three comparisons for each strain. The effect of extracts was determined by measuring the diameter of the mycelium with a digital vernier (Fisher Scientific, Waltham, MA, USA), the inhibition of each extract was calculated with the following equation:

$$\%Inhibition = \frac{Diameter\,of\,the\,sample\,colony - Inoculation\,diameter}{Diameter\,of\,the\,control\,colony} x100$$

Statistical analysis

A completely randomized-block experimental design was performed where the blocks corresponded to the fungus species. The inhibition of mycelium growth was analyzed by the analysis of variance (ANOVA) comparing the media by Tukey´s at α = 0.05. Terms were blocked in the analysis using the statistical package Minitab 17.

Results

Phytochemical screening

The methanolic extracts of bark and leaves of *J. platyphylla* were obtained in yields of 33.8 and 20.5% respectively, likewise the results of the preliminary phytochemical studies demonstrated the presence of pharmacologically important compounds, such as flavonoids, tannins, alkaloids, saponins, and terpenoids, which could account for the plant antimicrobial activities (Table 1).

HPTLC fingerprint profile

Flavonoid compound profile in *Jatropha platyphylla* methanolic extract: The HPTLC profile of *J. platyphylla* methanolic extract of bark and leaves showed the presence of flavonoids before and after derivatization. The *J. platyphylla* leaf extract showed the presence of up to 12 types of flavonoids at different Rf values in a range of 0.019–0.996 with colors that varied from yellow, blue, green, and orange (Table 3 and Fig. 1–2), while the bark extract had a number of up to eight flavonoids at Rf values that range from 0.23–0.97 and colors from blue to pale green (Table 4–5 and Fig. 1–2). As a control of the chromatography runs, three standards were used (naringin, chlorogenic acid, and apigenin 7-*O*-glucoside) (Table 2 and Fig. 1–2).

Terpenoid compound profile in *J. platyphylla* methanolic extract: The HPTLC fingerprinting for terpenoids was well resolved at UV 366 nm after derivatization. The plates were sprayed with anisaldehyde in sulfuric acid reagent followed by heating and then visualized in daylight, which showed bands from blue to violet colorations (Fig. 3–4), corresponding to terpenoid natural metabolites (Ramaiah and Garampalli 2015). The chromatograms obtained from the leaf extract revealed 12 peaks at different Rf values at a range of 0.044–0.967 (Table 6), while the bark showed the presence of seven peaks at Rf values of 0.003–0.967 (Table 7). As a control of the chromatography runs, diosgenin was used (Table 3–4 and Fig. 5).

Antifungal activity of *Jatropha platyphylla* methanolic extract: In general, the methanolic extracts from bark and leaf of *J. platyphylla* showed significant antifungal activity (*P < 0.05*) compared to the negative control with percentages of mycelial growth inhibition from 61.37–96.50% at concentrations of 0.05 and 0.2%, where the application of these extracts was associated with a decrease and deformation of colony growth. The colonies in the control grew with normal shape, size, and color after day 9 (Fig. 5–8).

The *J. platyphylla* bark and leaf methanolic extracts were observed to be highly effective in *C. cliviae* mycelial inhibition growth with percentages of inhibition of 96.50% even at concentrations of 0.05% of the extracts (Fig. 5). Significant statistical differences were recorded between the methanolic bark concentrations at 0.2 and 0.1%. However, no differences were found between the concentrations of 0.05 and 0.1% (Fig. 8). Likewise, the methanolic extract of *J. platyphylla* bark and leaves were statistically similar at the same concentrations against *C. cliviae* (Fig. 8).

In contrast, *F. oxysporum* f. spp. *lycopercisi* and *F. oxysporum radicis-lycopercisi* were less affected by the methanolic extracts of *J. platyphylla*, where 61.37–70.25% of the mycelial inhibition of *F. oxysporum* f. spp. *radicis-lycopercisi* and a 68.12–76.25% in the case of *F. oxysporum* f. spp. *lycopercisi* were achieved as shown in Fig. 6–8. In the case of *C. cliviae*, no significant difference was observed between the concentrations of bark and leaf methanolic extract evaluated against *Fusarium* spp. (Fig. 8). However, a greater efficacy of both extracts can be noted in mycelial growth inhibition of *F. oxysporum* f. spp. *lycopercisi*, where it grows amorphously when the extracts are included in the

Table 1: Phytochemical constituents of *J. platyphylla* leaf and bark methanolic extracts

Constituent	Test	MeOH *J. p* leaf	MeOH *J. p* bark
Tannins	FeCl₃	+++	++
	Gelatine	++	++
Flavonoids	Shinoda	+++	+
Alkaloids	Dragendorff	+	+
	Mayer	+	+
	Wagner	+	+
Saponins	Foam	++	++
Terpenoids	Salkowiski	+++	++
	Libermann	+++	++

(+++) Strong concentration; (+++) Medium concentration (+++); Low concentration; (-) Absent

Table 2: Standard chromatographic profile

Peaks	Rf	Height	Area	Substance
1	0.250	0.2428	0.0068	Naringin
2	0.384	0.2027	0.0085	Chlorogenic Acid
3	0.977	0.2709	0.0097	Apigenin 7-*O*-glucoside

Rf: Retention factor

Table 3: Chromatographic profile of *J. platyphylla* leaf ethanolic extract

Peaks	Rf	Height	Area	Substance
1	0.019	0.1216	0.0041	Flavonoid 1
2	0.073	0.0110	0.0002	Flavonoid 2
3	0.127	0.0891	0.0025	Flavonoid 3
4	0.180	0.2265	0.0069	Flavonoid 4
5	0.271	0.1189	0.0031	Flavonoid 5
6	0.310	0.5428	0.0398	Flavonoid 6
7	0.484	0.5603	0.0452	Flavonoid 7
8	0.659	0.0110	0.0044	Flavonoid 8
9	0.824	0.0261	0.0009	Flavonoid 9
10	0.853	0.0201	0.0002	Flavonoid 10
11	0.977	0.0367	0.0004	Apigenin 7-*O*-glucoside
12	0.996	0.0267	0.0002	Flavonoid 12

Rf: Retention factor

Table 4: Chromatographic profile of *J. platyphylla* bark methanolic extracts

Peaks	Rf	Height	Area	Substance
1	0.230	0.0205	0.0004	Flavonoid 1
2	0.329	0.0068	0.0002	Flavonoid 2
3	0.394	0.0194	0.0011	Flavonoid 3
4	0.517	0.0388	0.0016	Flavonoid 4
5	0.557	0.0315	0.0008	Flavonoid 5
6	0.653	0.0088	0.0004	Flavonoid 6
7	0.780	0.0864	0.0033	Flavonoid 7
8	0.976	0.2169	0.0053	Apigenin 7-*O*-glucoside

Rf: Retention factor

Table 5: Standard chromatographic profile

Peaks	Rf	Height	Area	Substance
1	0.441	0.2179	0.0083	Diosgenine

Rf: Retention factor

medium compared to *F. oxysporum* f. spp. *radicis-lycopercisi*, whose mycelium grows uniformly, thus showing a slightly higher susceptibility (Fig. 6–7).
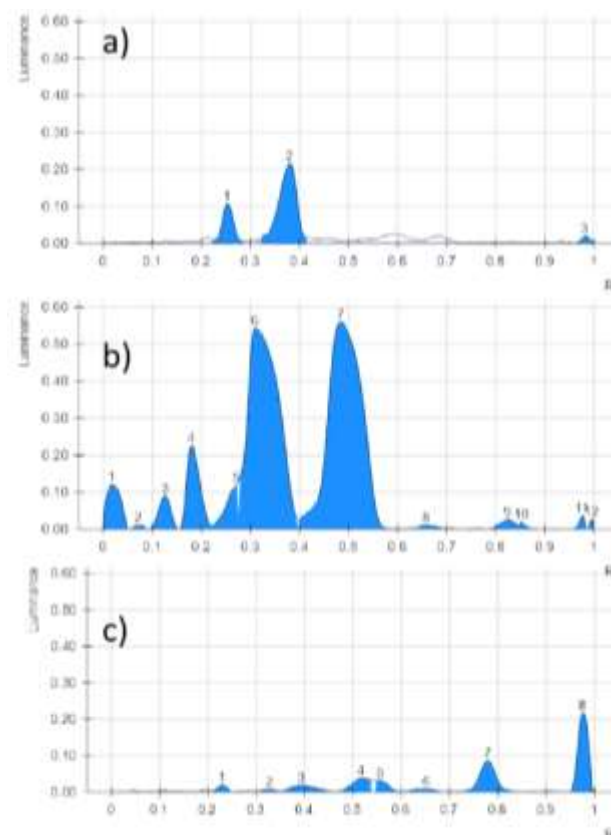

Fig. 1: High-performance thin layer liquid chromatography (HPTLC) fingerprint chromatogram for flavonoids; (**a**) standard; (**b**) *J. platyphylla* leaf methanolic extract; (**c**) *J. platyphylla* bark methanolic extract
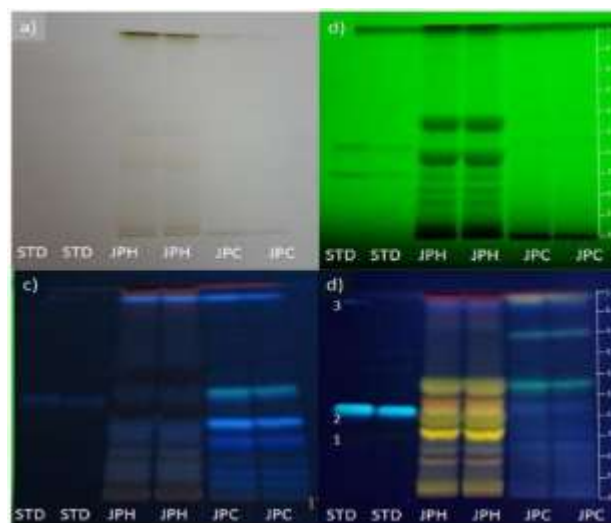

Fig. 2: High-performance thin layer liquid chromatography (HPTLC) fingerprint profile for flavonoids of standard of naringin, chlorogenic acid, and apigenin 7-O-glucoside (STD); *J. platyphylla* leaf methanolic extract (JPH); *J. platyphylla* bark methanolic extract (JPC) at: (**a**) daylight; (**b**) 254 nm; (**c**) 365 nm; (**d**) 365 nm after derivatization 2-aminoethyl diphenylborinate at 98% and 400 polyethylene glycol
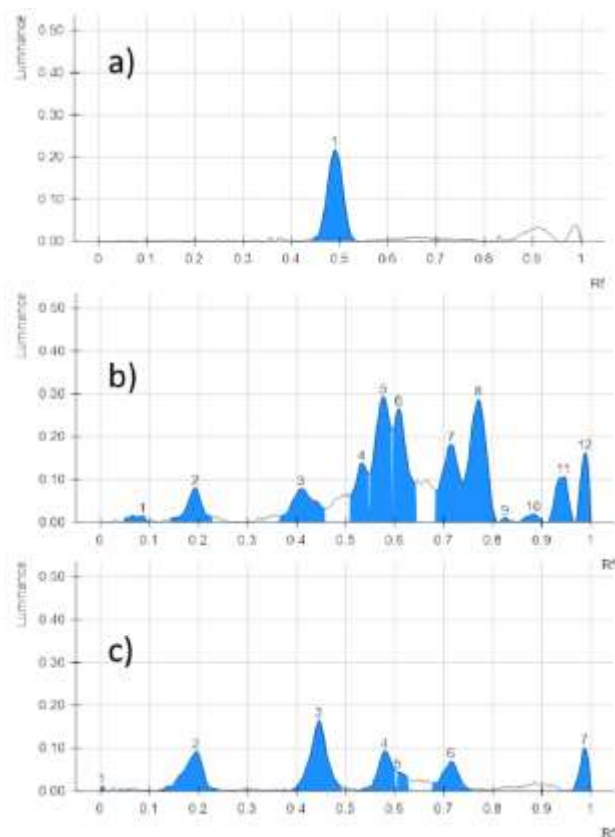
Fig. 3: High-performance thin layer liquid chromatography (HPTLC) fingerprint chromatogram for terpenoids; (**a**) standard; (**b**) leaf methanolic extract of *J. platyphylla*; (**c**) bark methanolic extract of *J. platyphylla*
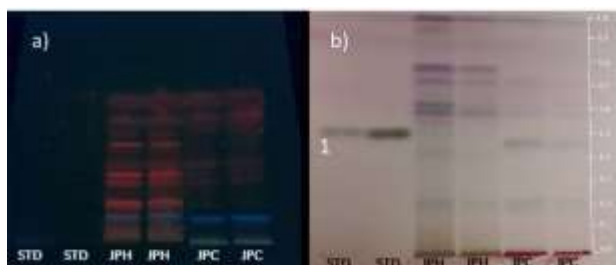

Fig. 4: High-performance thin layer liquid chromatography (HPTLC) fingerprint profile for terpenoids of diosgenin standard (STD); *J. platyphylla* leaf methanolic extract (JPH); *J. platyphylla* bark methanolic extract (JPC) at: (**a**) 365 nm; (**b**) daylight after derivatization with anisaldehyde in sulfuric acid

Discussion

This study showed that *J. platyphylla* methanolic extracts from bark and leaves have a pronounced antifungal activity against *C. cliviae, F. oxysporum* f. spp. *lycopersici* and *F. oxysporum* f. spp. *radicis-lycopersici* and may be considered sources of bioactive phytochemicals. Although not many studies are related to the antifungal activities of *J.*

Table 6: Chromatographic profile of *J. platyphylla* leaf ethanolic extract

Peaks	Rf	Height	Area	Substance
1	0.044	0.0159	0.0006	Terpenoid
2	0.144	0.0804	0.0029	Terpenoid
3	0.367	0.0786	0.0044	Terpenoid
4	0.509	0.1386	0.0042	Terpenoid
5	0.549	0.2931	0.0104	Terpenoid
6	0.596	0.2647	0.0084	Terpenoid
7	0.684	0.1814	0.0072	Terpenoid
8	0.739	0.2875	0.0113	Terpenoid
9	0.810	0.0107	0.0002	Terpenoid
10	0.850	0.0193	0.0006	Terpenoid
11	0.911	0.1068	0.0032	Terpenoid
12	0.967	0.1628	0.0030	Terpenoid

Rf: Retention factor

Table 7: Chromatographic profile of *J. platyphylla* bark methanolic extracts

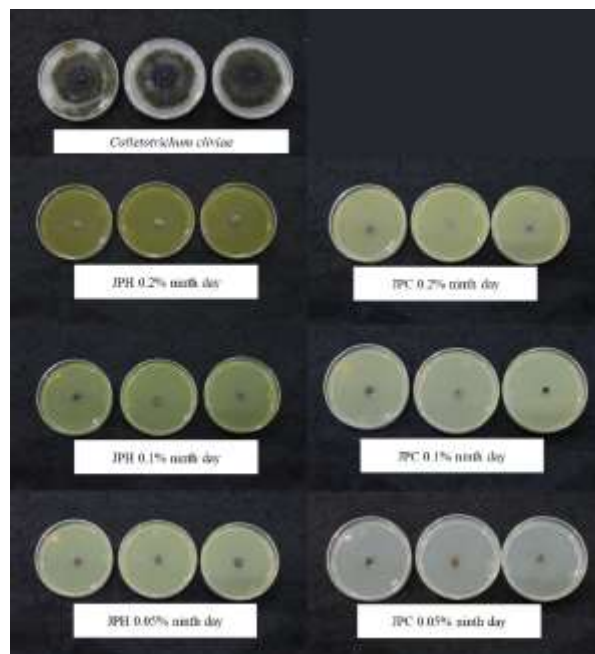| Peaks	Rf	Height	Area	Substance
1	0.001	0.0108	0.0001	Terpenoid
2	0.114	0.0922	0.0042	Terpenoid
3	0.367	0.1655	0.0067	Terpenoid
4	0.533	0.0931	0.0031	Terpenoid
5	0.606	0.0448	0.0008	Terpenoid
6	0.677	0.0697	0.0027	Terpenoid
7	0.961	0.1008	0.0020	Terpenoid

Rf: Retention factor

Fig. 5: Growth inhibition zone (mm) of *Colletotrichum cliviae* after incubation for nine days at 37°C on potato dextrose agar medium containing *J. platyphylla* extracts from bark (JPC) and leaves (JPH)

platyphylla, several reports of extracts from species of the *Jatropha* genus have demonstrated their ability to exert antifungal activity. Rahman *et al.* (2011) reported that the methanolic extract of *J. curcas* seed at a concentration of 10
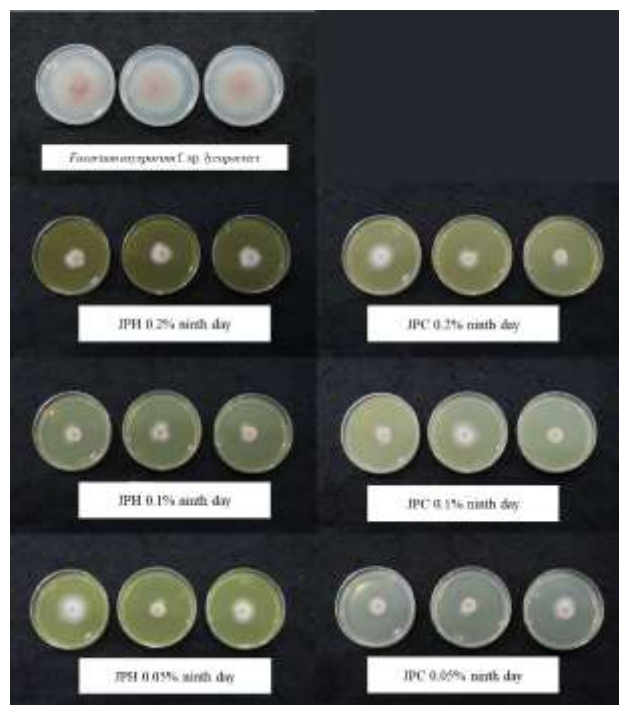
Fig. 6: Growth inhibition zone (mm) of *F. oxysporum* f. spp. *lycopersici* after incubation at 37°C on potato dextrose agar medium containing *J. platyphylla* extracts from bark (JPC) and leaves (JPH) for nine days

Fig. 7: Growth inhibition zone (mm) of *F. oxysporum* f. spp. *radicis-lycopersici* after incubation at 37°C on potato dextrose agar medium containing *J. platyphylla* extracts from bark (JPC) and leaves (JPH) for nine days
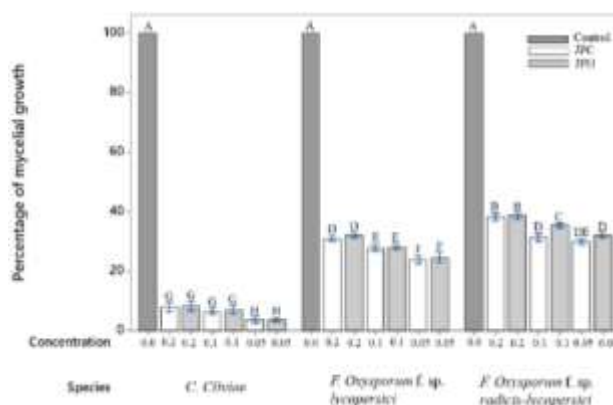

Fig. 8: Effect of the amount of methanolic extract of *J. platyphylla* bark and leaves on *C. clivae musae* growth by the agar dilution method. Values are means of three replicates. Significant differences among treatments at *P < 0.05* are indicated with a letter as measured by Tukey's test

mg/mL was effective controlling 78.87% of *C. gloeosporioides* mycelial growth until day seven, while the fruit extract only inhibited 46.42% of mycelial growth at the same concentration. Similarly, Saetae and Suntornsuk (2010) demonstrated that *J. curcas* seed crude extract inhibited 100% of *C. capsici* mycelial growth at 4 mg mL^{-1}. These concentrations can be considered high, since *J. platyphylla* extracts of bark and leaves were effective in inhibiting up to 96.50% at concentrations of 0.5 mg mL^{-1} even after nine days. This variation in activity may be due to the difference in the groups of active compounds reported in *J. curcas* fruit cake and seeds, which are mainly proteins, fats and phorbol esters found in these extracts (Saetae and Suntornsuk 2010; Rahman *et al.* 2011). On the other hand, the *J. platyphylla* species develops in the wild in high humidity environments (Makkar *et al.* 2011), which facilitates fungal growth, which could be forcing the plant to constantly interact with a high variety of fungi. Over the years, this natural interaction could have induced an evolutionary defense response against phytopathogenic fungi, promoting the development of a wide variety of secondary metabolites in the most exposed *J. platyphylla* organs, such as the leaf and stem, which could explain the variation in activity among the extracts from different species (Hiruma 2019).

Similarly, previous works have reported partial inhibition of *Fusarium* species mycelial growth using *J. curcas* oils. However, in the case of *Colletotrichum*, the necessary concentrations are high as reported by Cordova-Albores *et al.* (2014), a decrease in mycelial growth rate of 0.77 cm per day was observed when 2.5 mg/mL concentrations of *J. curcas* seed oil in PDA were used against *F. oxysporum* f. spp. *gladioli*, suggesting a fungistatic effect. In a subsequent study, since *J. curcas* oil from seed can cause changes in the morphology of the

external cover of the mycelium and conidia, as well as inhibition of metabolic processes, the fungistatic effect on *F. solani* has been possible, which was verified by different microscopic and fluorochromatic techniques (Cordova-Albores *et al.* 2016). These results are similar to those obtained in this research with *J. platyphylla* extracts, which despite a decrease in mycelial growth of up to 76.25% at 0.2 mg mL^{-1} and the observed modification of its morphology, it did not prevent its growth, determining the fungistatic potential of the metabolites present in *J. platyphylla* on the evaluated *F. oxysporum* species.

Because quality of plant extracts and their biological properties depend on the presence of active phytoconstituents in their organs, phytochemical analyses were performed on the methanolic extracts evaluated. The results indicated that *J. platyphylla* bark and leaves are rich in a wide variety of secondary metabolite groups, such as flavonoids, tannins, saponins, alkaloids and terpenoids. *J. platyphylla* HPTLC fingerprint profile revealed a total of 12 spots for leaves and eight for bark, using 2-aminoethyl diphenylborinate at 98% and 400 polyethylene glycol derivatizing agents for flavonoids. Jug *et al.* (2018) reported that the use of these reagents provides a wide diversity of color fluorescence bands for different flavonoids at 366 nm, such as green (kaempferide, apigenin, naringenin, pinocembrin, kaempferol) orange (quercetin dihydrate, myricetin, chrysin), blue for some phenolic acids (chlorogenic acid, rosmarinic acid, caffeic acid) and yellow (luteolin). In this sense, the *J. platyphylla* extracts show a great variety of flavonoids in their profile, previously reported by Ambriz-Pérez *et al.* (2016), where 17 phenolic compounds were detected in phenolic extracts from *J. platyphylla* leaf and fruit, most of them were apigenin, genistein and luteolin glycosides. Moreover, apigenin and luteolin glycosides have been found in other *Jatropha* species, such as *J. curcas* (Abd-Alla *et al.* 2009), *J. multifida* (Moharram *et al.* 2007) and *J. gossypifolia* (Mariz *et al.* 2010).

Additionally, as shown in the chromatograms, one of the spots between the leaf and bark extracts showed similar Rf and color characteristic to the apigenin 7-*O*-glucoside standard (leaf Rf: 0.979, blue color, bark Rf: 0.979, blue color and to the apigenin 7-*O*-glucoside Rf: 0.977, blue color) (Table 2–4 and Fig. 1–2). In this sense, the extract from leaf and bark could be assumed to contain the apigenin 7-*O*-glucoside, which has been related to its antifungal activity on *Candida* spp. (Smiljkovic *et al.* 2017). Flavonoids have been widely associated with a large range of biological activities, highlighting its antimicrobial activity (Kanwal *et al.* 2009, 2010; Górniak *et al.* 2019). The inherent antimicrobial properties of these compounds have been reported to be determined by their chemical structure since in ring A, hydroxylations at positions 5 and 7 are the most critical for flavonoids antimicrobial activity (Cushnie and Lamb 2011; Yang *et al.* 2017). Furthermore, because its capacity of inhibiting fungal growth had been reported with various underlying mechanisms, including plasma membrane disruption, the mitochondrial dysfunction induction and inhibiting the following: cell wall formation, cell division, RNA and protein synthesis, and the efflux mediated pumping system (Al-Aboody and Mickymaray 2020).

While the terpene profile in *J. platyhylla* methanolic extracts shows the presence of 12 different terpenes in the leaf and seven in the bark, different types of terpenes have been previously reported in *Jatropha* species, including curcusone B and stigmasterol from the stem bark of *J. curcas* and jatrophone from the stem bark of *J. gossypifolia*, which showed great antibacterial activity against *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* (Sahidin *et al.* 2012). Additionally, two diterpenes (jatrophone and jatropholone B) and a triterpene (9,13-dihydroxyisabellione) were isolated from *J. isabelli* rhizomes (Pertino *et al.* 2007). These compounds have been reported as typical constituents of several Euphorbiaceae, including *J. gossypifolia* (Purushothaman *et al.* 1979). Thus, the presence of a great diversity of terpenes in both extracts is of great interest because this group of metabolites is associated with potent antifungal activity since they can change the microbial cell membrane properties and functions because of increasing membrane fluidity observed under exposure (Tao *et al.* 2019). Alterations in membrane permeability vary according to the concentration since high concentrations cause severe damage and loss of homeostasis (Scariot *et al.* 2021). Likewise, some terpenoid components interfere with the amino acid involved in spore germination and denature the enzymes responsible for germination, energy production and synthesis of structural compounds (Castro *et al.* 2020).

Conclusion

This study shows that methanolic extracts from *J. platyphylla* bark and leaf can act as a strong fungicide by inhibiting the mycelial growth of *C. cliviae* by up to 96.50, 70.25 and 76.25% and *F. oxysporum lycopercisi* 76.25% at a concentration range of 0.2–0.05%. The phytochemistry essay showed the presence of tannins, flavonoids, alkaloids, saponins, terpenoids, and alkaloids. The HPTLC analysis reports the presence of apigenin 7-*O*-glucoside from *J. platyphylla* bark and leaves; moreover, the fingerprint profile from the extracts confirmed at least 12 different types of flavonoids in leaves, and eight in bark, as well as 12 different types of terpenoids in leaves and seven in barks, which could have contributed to its antifungal activity. As a perspective, the purification of these groups of molecules should be performed to determine their role in antifungal activity.

Acknowledgments

Author Contributions

MA planned the experiment and made the write up, IM planned the experiment, RS, FS, JM, and MA interpreted the results and made editorial corrections.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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