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Managing Brown Rot Disease of Citrus Fruit Using Plant Extracts

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ABSTRACT

Brown rot, caused by *Phytophthora nicotianae* and *P. citrophthora*, is a major disease of citrus fruit, leading to significant economic losses globally. Conventional fungicides are commonly used to manage this disease, but concerns have been raised due to environmental persistence, human toxicity and the emergence of resistant strains. This study evaluated the spore germination and mycelial growth inhibition of both *Phytophthora* species by extracts of 31 plant species in vitro and in vivo. DCM/MeOH extracts were tested against both pathogens using a microtiter plate assay and an amended medium assay. All bioactive extracts were further fractionated into seven fractions using solid phase extraction (SPE), which were subsequently tested against both pathogens. Phytochemical profiling was determined by ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC–QTOF-MS) analysis. Extracts of seven plants (*Artemisia afra*, *Dombeya rotundifolia*, *Eucomis regia*, *Olea europaea*, *Cannabis sativa*, *Peltophorum africanum* and *Mentha longifolia*) inhibited both *P. nicotianae* and *P. citrophthora* with a MIC < 1 mg/mL and MFC < 2.5 mg/mL as compared to azoxystrobin (6.0 mg/mL). In the lemon fruit in vivo assays, *A. afra* reduced brown rot severity by > 50% compared to untreated controls, with superior performance to azoxystrobin, the standard fungicide. UPLC–QTOF-MS profiling of bio-active fractions identified scopoletin (*A. afra*), oleuropeinic acid (*O. europaea*) and bergenin (*P. africanum*) as putative contributors to the observed activity. These findings highlight *A. afra* as a promising natural product and suggest that other extracts warrant further evaluation for use as alternative oomyceticides in citrus post-harvest protection.

1 | Introduction

Citrus farming is a significant economic driver in many countries, including South Africa. It is one of South Africa's top exports, with an exportation value of about 171 million cartons, generating an annual revenue of R30 billion (Chisoro and Roberts 2024). Citrus production also makes a substantial contribution to other agricultural economic sectors, such as the transformation industry, research development and job creation. There is a need for improved plant disease management in line with the EU Green Deal strategies to preserve food security and safety (World Health Organization 2018; Sarrocco and Vannacci 2018).

Phytophthora nicotianae and *P. citrophthora* are the most common *Phytophthora* species linked to citrus brown rot (Ippolito et al. 2004; Watanarojanaporn et al. 2011). They are oomycetes, fungal-like pathogens that can cause citrus brown rot

and reduce orchard productivity, causing significant financial losses (Adaskaveg et al. 2024). The pathogens infect the roots, stems, branches, leaves, twigs and fruits of citrus trees, causing fruit brown rot, canopy blight, root rot, foot rot, gummosis and damping-off of seedlings (Cacciola and di Magnano San Lio 2008). Fruit infection leads to dark, water-soaked lesions that spread quickly and cause fruit deterioration (Graham and Feichtenberger 2015). Water, soil and diseased plant debris often disperse these pathogens, which thrive in warm, humid environments (Etebu and Nwauzoma 2014).

The current methods of managing brown rot include orchard management and the use of synthetic fungicides such as copper hydroxide, copper oxychloride, fosetyl-aluminium, mancozeb and metalaxyl (Pscheidt 2023). Copper fungicides have been used extensively used in citrus brown rot management; they provide protection and eliminate sporangia on the fruits. One major drawback is short term protection leading to frequent chemical reapplication (Albrigo et al. 1997; Behlau et al. 2017; Diepenbrock et al. 2025). Extensive use of fungicides can lead to pollution of water and soil, elimination of beneficial soil microorganisms and an increase in the occurrence of resistant strains (Kenarova and Boteva 2023). *Phytophthora* spp. are known to develop resistance to fungicides due to their genetic flexibility, overexpression of target genes and mutation of target sites (Gonzalez-Tobon et al. 2022). A study conducted by Wang et al. (2020) on *P. capsici* and *P. infestans* revealed that mutations of the RPA190 gene, similar to those found in *P. nicotianae* and *P. citrophthora*, have been linked to metalaxyl resistance, which is of great concern (Van Niekerk et al. 2019).

Research has been conducted to identify biological control agents (BCAs) effective against plant pathogens, with various modes of action, low toxicity and easy degradation, thereby reducing the reliance of agricultural production on toxic synthetic pesticides (Cantrell et al. 2012; Zhengqi et al. 2023). Several medicinal plants with known antimicrobial potential have been successfully used against plant pathogens (Hermann et al. 2022). However, very few studies have comprehensively screened plant extracts for efficacy against citrus-infecting *Phytophthora* spp., particularly *P. nicotianae* and *P. citrophthora*.

The use of plant-based pesticides faces several challenges, such as standardisation, lower efficacy, the need for frequent application and the durability of the formulated products (Lyousfi et al. 2022). However, despite these challenges, some plant-based compounds, such as neem (*Azadirachta indica*), pyrethrin (*Tanacetum cinerariifolium*) (Mahmoud et al. 2011; Sharafzadeh 2011) and rotenone from *Derris* and *Lonchocarpus* spp. (Sola et al. 2014), have been manufactured and sold for use as insecticides. The aim of this study was to screen 31 plant extracts and their corresponding pre-fractionated extracts for their ability to inhibit the mycelium growth and zoospore germination of *Phytophthora nicotianae* and *P. citrophthora* for post-harvest treatments of citrus fruit in vitro. Thereafter, the bioactive compounds of selected extracts were identified using UPLC-QTOF-MS. The best-performing extract was further evaluated for its in vivo efficacy against the *Phytophthora* species using lemon fruit.

2 | Materials and Methods

2.1 | Plant Selection and Collection

Plant materials comprising leaves, entire plants, roots, twigs and combinations of leaves and flowers were used in this research. The plant materials were obtained from the repository of plant samples of the Biodiscovery Centre, Department of Chemistry, University of Pretoria. Relevant authorities in South Africa granted permission to collect the plant material in the repository, and the voucher specimens were deposited at the South African National Biodiversity Institute, Pretoria (SANBI), as shown in Table S1. The plants used in this study were selected based on an online search of literature where the following keywords were used: 'citrus', 'Phytophthora inhibition', 'brown rot', 'traditional medicinal plants' and 'biocontrol agents'. A scoring method was used to prioritise the selected plants, and the criteria were as follows:

(a) Traditional use of plants in the treatment of *Phytophthora* diseases or brown rot was given a score of 3; plants with potential against fungal pathogens were scored 2, while score 1 was given to plants with other traditional uses.

(b) Plant parts used: leaves with a score of 3, stems and barks with a score of 2 and roots with a score of 1.

(c) Strength of the published data; plants with two or more published articles related to antimicrobial properties were scored 3; plants with fewer publications scored 2, while a score of 1 was given to plants without any published information. Plants with the highest scores were finally selected (Fouche et al. 2011; Mianda et al. 2022).

2.2 | Extraction and Fractionation of Plant Material

Plant samples were extracted using a glass extraction vessel filled with approximately 7 g of air-dried ground plant material. The vessel containing this material was filled with 50 mL of a 1:1 mixture of dichloromethane (DCM) and methanol (MeOH), and it was sonicated for 1 h at 38°C in an ultrasonic bath (Invernizzi et al. 2022). The second extraction was carried out using 50 mL of MeOH; the solution was drained and collected into round-bottom flasks. The SP Genevac HT6 (Genevac Ltd., Ipswich, UK) was used to dry the combined DCM/MeOH and MeOH extracts. Extracts were fractionated using a modified version of the National Cancer Institute (NCI) methodology by Thornburg et al. (2018). Each extract was fractionated into seven fractions using a Gilson GX-241 ASPECR liquid handler and a HypeSep C8 SPE cartridge (2 g/6 mL). The combined DCM/MeOH and MeOH extracts (250 mg) were adsorbed onto a cottonwool roll after being dissolved in 5 mL of a 6:3:1 solution of MeOH, ethyl acetate and methyl tert-butyl ether (manual liquid handling). After drying, the cottonwool roll containing the crude extract was placed inside a 10 mL SPE cartridge. Fractionation was initiated as previously described by Invernizzi et al. (2022), with seven fractions (designated as f1 to f7) collected for each extract. The

eluent system consisted of water, MeOH and ACN (purchased from Microsep, Gauteng, South Africa). The fractions were collected as 19:1 (H_2O: MeOH); 4:1 (H_2O: MeOH); 3:2 (H_2O: MeOH); 1:1 (H_2O: MeOH); 2:3 (H_2O: MeOH); 100% (MeOH); and 1:1 (ACN: MeOH). The SP Genevac HT6 was used to dry the collected fractions. Prior to screening, the resultant dried fractions and extracts were placed in a freezer at 4°C.

2.3 | Preparation of Ethanol Extract of Artemisia Afra

Leaves and stems of *Artemisia afra* were harvested from the Agricultural Research Council, Roodeplaat, Pretoria, South Africa (ARC-LSP), in March 2025. A voucher specimen (PRU 121389) was prepared and deposited at the University of Pretoria's H.G.W.J. Schweickerdt Herbarium. *Artemisia afra* leaves and stems were rinsed with clean water, air-dried and ground into fine powder. Of this fine powder, an aliquot of 100 g was soaked in 1000 mL ethanol, and the mixture was left on an orbital shaker for 24 h. The resultant ethanol solution, which is considered to be occupationally safe and less toxic than DCM/MeOH, was filtered and collected into a round-bottom flask. An SP Genevac HT6 (Genevac Ltd., Ipswich, UK) was used to dry the ethanol extract, which was stored at 5°C for future use.

2.4 | UPLC–QTOF-MS Analysis of Artemisia Afra, *Olea europaea* and Peltophorum Africanum

A Waters ACQUITY UPLC system (Waters Corp., MA, USA) was used to perform ultra-high-performance liquid chromatography (UPLC) analysis of crude extracts and the corresponding SPE-generated fractions. A small centrifuge was used to spin down the crude extracts and fractions for 30 s after dissolving in MeOH (Methanol 215, Super Purity, Romil, Waterbeach, England). Waters ACQUITY UPLC BEH C18 (2.1 × 100 mm, 1.7 μm) column (Waters Inc., Milford, MA, USA) was used for the separation of compounds in the samples. The dry dichloromethane/methanol extract and fractions were dissolved in methanol/water (1:1 ratio) to a concentration of 1 mg/mL, sonicated for 10 min at ambient temperature, followed by filtration through a 0.22-μm syringe filter. Analyses were carried out using a Waters ACQUITY UPLC HSS T3 (2.1 × 150 mm, 1.8 μm) column (Waters Inc., Milford, MA, USA), showing compounds with the best selectivity. During separation, the solvents used were water (0.1% FA, 0.01% ammonium formate) and methanol (0.1% FA). This separation method has the following conditions: 97% water (0.1% formic acid), stabilised for 0.1 min, thereafter increased linearly to 100% methanol at 14 min. Before reconditioning the column, a 2-min washing hold period was observed (14–16 min). For the consistency of the results, a 0.3 mL/min flow rate, a 5 μL injection volume and a 40°C temperature were maintained (Invernizzi et al. 2022). For compound identification, the data obtained were analysed in both positive and negative mode electrospray ionisation. The molecular formulae of the compounds obtained from Masslynx based on their MS/MS fragmentation, mass error and %Fit Confidence were compared to suggested compounds from online databases such as PubChem, Lotus, KNApSAcK and Waters UNIFI Scientific Information System (version 1.9.2). The accurate masses obtained were compared to those of known compounds previously reported for the genera *Olea*, *Artemisia* and *Peltophorum*, and MS fragmentation patterns were used for compound identification.

2.5 | Zoospore Production

Two pathogenic *Phytophthora* species, viz. *P. nicotianae* (P. 121, P. 137, P. 1395 and P. 1459 strains) and *P. citrophthora* (P. 2476, P. 1988, P. 1465 and P. 2534 strains), known to cause brown rot on citrus fruits were obtained from Citrus Research International (CRI), Nelspruit. The isolates were maintained on PARPH agar-modified plates as described by Graham et al. (1998). For zoospore production, the isolates were grown on 10% clarified V8 (V8C) juice agar for 48 h in the dark at 28°C and subsequently incubated at room temperature (25°C) for 48 h to enhance mycelium production. To induce sporangia production, 10 plugs (5 mm diameter) of 7-day-old cultures were placed in a Petri dish flooded with 15 mL of a 1.5% soil extract solution (SES), incubated at 25°C for 48 h in the dark, followed by incubation under fluorescent light for 24 h. Zoospore production was induced by incubating the plates at 4°C for 20 min and then at 25°C as described by Adaskaveg et al. (2015). After 30–60 min of incubation, the zoospore suspension was adjusted to the appropriate concentration (1×10^6 spores/mL) using a haemocytometer (0.100 mm–0.0025 mm^2) (Germany) and an Optika microscope (Ceti Microscopes 6Vdc 1000 mA-XLEDB-293 Chalgrove, Ponteranica, Italy) (Lyousfi et al. 2022).

2.6 | In Vitro Effect of DCM/MeOH Extracts on the Mycelial Growth of *P. nicotianae* and *P. citrophthora* Using MIC

The lowest extract concentration that prevents microbial growth is known as the minimum inhibitory concentration (MIC). The MIC of a reference fungicide (azoxystrobin), crude extracts and fractions was determined using the Eloff (1998) method, modified for antimicrobial testing by Masoko et al. (2005). Each assay was performed in triplicate, and the experiment was repeated. The various extract residues were dissolved in DMSO: V8 broth (1:39) to produce a stock solution with a concentration of 2.5 mg/mL. Thereafter, 100 μL of V8 broth were pipetted into each well, followed by 100 μL of plant extract (Eloff 1998). Subsequently, 100 μL of a zoospore suspension (1×10^6 spores/mL) were added to each well. Azoxystrobin (6.0 mg/mL) was used as the positive control, and DMSO: V8 broth (1:39) was used as the negative control. The 96-well plate was placed in a plastic bag to reduce fungal contamination and then incubated for 48–72 h at 25°C and at 95% relative humidity. P-iodonitrotetrazolium violet (INT), a growth indicator, was prepared at a concentration of 0.2 mg/mL and 40 μL of this solution were added to each microtitre well. Biologically active organisms convert the colourless tetrazolium salt to a red formazan product, which serves as an electron acceptor (Eloff 1998). After being incubated with P-iodonitrotetrazolium violet (INT), the solution in the well either stays clear or exhibits a visible decrease in

colour intensity, indicating suppression of microbial growth. A ThermoFisher Spectrophotometer (24 VDC/4 A, Vantaa, Finland) was used to measure absorbance at 590 nm.

$$MIC = C_o \div D,$$

where C_o = initial highest concentration of the antimicrobial agent; D = dilution factor at the well with no growth (for 10-fold, $D = 10^n$).

2.7 | In Vitro Effect of DCM/MeOH Extracts on Phytophthora Mycelial Growth

The seven plant extracts with the lowest MIC values identified in Section 2.6 were tested for their ability to inhibit the mycelium growth of both *P. nicotianae* and *P. citrophthora* using the amended medium assay. The plant extracts were tested at concentrations of 1.25 and 2.5 mg/mL. In addition, the most effective plant extract (*A. afra*) identified through the MIC study (Section 2.6) was also tested at concentrations of 1.25, 2.5 and 5 mg/mL against the six virulent *Phytophthora* isolates. PDA medium was allowed to cool before the extract was added to avoid compound degradation. A mycelium plug (5 mm in diameter) of each strain was extracted aseptically from 1-week-old cultures growing on V8 agar. These plugs were placed on Petri dishes containing PDA amended with different extract concentrations for all treatments, while the negative and positive controls consisted of an unamended PDA and azoxystrobin (6.0 mg/mL), respectively (El Khetabi et al. 2020; Talibi et al. 2012). After being sealed with parafilm, the Petri plates were incubated for 7 days at 25°C. By measuring the diameter (mm) of microbial growth using a Vernier calliper, the in vitro effect of each plant extract was expressed as the inhibition percentage when compared to the control (Lahlali et al. 2020). The following formula was used to determine the mycelium growth inhibition (MGI):

$$MGI\,(\%) = [(\text{control diameter} - \text{treatment diameter})/\text{control diameter}] \times 100.$$

The experiment was conducted three times, with each treatment undertaken in triplicate.

2.8 | Fruit Inoculations for Determining the Curative and/or Preventive Traits of the Ethanol Artemisia Afra Extract

In the curative trials, detached lemon fruit was wounded at two positions, perpendicular to the stem end. The fruit flavedo was removed with 60-grit autoclaved sandpaper (1 cm^2) to create superficial lesions without penetrating the albedo (3 mm depth; 4 mm diameter). A sterile piece of miracloth (1 cm^2) dipped in a zoospore suspension (1×10^6 spores/mL) was applied to the wounds. The miracloth was removed once it had dried. Twenty-four hours after inoculation, the fruits were dip-treated separately with azoxystrobin, dimethyl sulfoxide/sterilised distilled water (DMSO/SDW (1:39)) and different concentrations (1.25, 2.5 and 5 mg/mL) of the ethanol *A. afra* extract. The fruits used in the preventive trials were first wounded as previously mentioned, treated with the appropriate fungicides, extracts and allowed to dry. A sterile piece of miracloth (1 cm^2) dipped in a zoospore suspension (1×10^6 spores/mL) was applied to the wounds 24 h later (El Khetabi et al. 2020; Lahlali et al. 2020; Merwe et al. 2023).

2.9 | In Vivo Effects of the Ethanol Artemisia Afra Extract on Citrus Brown Rot

Based on the in vitro screening results, DCM/MeOH extracts of *A. afra* showed the highest significant effect on the tested pathogens; therefore, it was selected for the in vivo trial. Ethanol, which is less toxic and a safer solvent to handle than DCM/MeOH, was used to prepare the *A. afra* extract, extract was dissolved in DMSO/SDW (1:39 ratio) to concentrations of 1.25, 2.5 and 5 mg/mL. Thirty detached Eureka lemon fruits were rinsed with double autoclaved distilled water (ddH_2O), disinfected in 1% bleach for 5 min and rinsed again with ddH_2O for 5 min. Treatment was carried out by dipping the fruits in the *A. afra* extract at 1.25, 2.5 and 5 mg/mL concentrations, under the same conditions, the negative control was treated with the same volume of DMSO/SDW (1:39), while azoxystrobin (6.0 mg/mL) was used as the positive control. After 24 h of incubation at room temperature, the treated fruits were inoculated with zoospore suspensions of *P. nicotianae* and *P. citrophthora* at a concentration of 1×10^6 spores/mL as described above. Thereafter, all the fruits were placed in incubation crates at 25°C with ice cube trays filled with 2% soil broth to maintain high humidity (Askarne et al. 2013; Lahlali et al. 2020). Lesion diameter was measured with a Vernier calliper after 7 days of incubation, and the disease severity was determined for each treatment (*A. afra* extract, DMSO/SDW (1:39) and azoxystrobin control) as follows: The proportion of area or amount of plant tissue that is diseased and is usually expressed as a percentage or proportion of plant area or fruit volume destroyed by a pathogen.

$$\text{Disease severity or Infection index} = \frac{\text{Sum of all disease rating} \times 100}{\text{Total no. of rating} \times \text{Maximum disease score}}$$

2.10 | Statistical Analyses

RStudio was used to statistically analyse the data (R software version 4.3.0; Wickham and Bryan 2023). Depending on the dataset structure, either a one-way or two-way ANOVA was performed. Tukey's HSD was then employed as a post hoc test to assess statistical differences between treatments (p value < 0.05). All results are reported as mean ± SD unless otherwise stated.

3 | Results

3.1 | Plant Selection and Collection

The use of a preferential plant scoring system modelled on the traditional uses of plant species improved the selection process by refining the pool of possible plants to only those with the potential to inhibit oomycetes. Initially, 58 medicinal plant species were selected. Selection was based on the highest priority scores, and a scoring system was used to prioritise the selected plants: a score of 3 represented strong, 2 medium and 1 weak. A total score of 6 was used to rank plants; 31 plant species were deemed

suitable for investigation, as they scored greater than 6, as shown in Table S1. This table provides information on the plant species, voucher number, family name, common name, traditional use, plant part used, reported published data, GPS coord collected and the final score given to the selected plant species.

3.2 | Extraction and Fractionation of Plant Material

Sequential ultrasonic bath extraction of dried and macerated samples of the 31 plant materials resulted in extracts, with the extraction yields given in Table S1. All the plant extracts were evaluated for their antimicrobial inhibitory activity against zoospores of both *Phytophthora* species (Table 1a,b). Based on the MIC values of less than 1 mg/mL, the extracts from *Artemisia afra*, *Olea europaea*, *Dombeya rotundifolia*, *Eucomis regia*, *Mentha longifolia*, *Peltophorum africanum* and *Cannabis sativa* were selected for further fractionation. DCM/MeOH extracts from these plants were fractionated using the SPE, which resulted in seven fractions per plant extract, generating a total of 49 different SPE fractions. Separately, the extraction of 1.52 kg of dried *Artemisia afra* leaves and stems using ethanol yielded 92.13 g of dry crude ethanol extract, which was used for the in vivo trials.

3.3 | In Vitro Effect of DCM/MeOH Extracts on *P. nicotianae* and *P. citrophthora* Using MIC

The results of the in vitro inhibition of zoospore germination and mycelium growth of *P. nicotianae* by the 31 selected plant extracts are shown in Table 1a. A value < 1 mg/mL was selected as a threshold for the MIC, as values ranging from 0.1 to 1 mg/mL are considered indicative of anti-oomycete activity against *Phytophthora*. Of the 31 screened plants, extracts of *Artemisia afra* (MIC 0.274 mg/mL), *Olea europaea* (MIC 0.801 mg/mL), *Dombeya rotundifolia* (MIC 0.896 mg/mL), *Eucomis regia* (MIC 0.860 mg/mL), *Mentha longifolia* (MIC 0.703 mg/mL), *Peltophorum africanum* (MIC 0.547 mg/mL) and *Cannabis sativa* (MIC 0.547 mg/mL) showed high inhibitory activity against *P. nicotianae*.

Similarly, Table 1b shows the results of the 31 plant extracts against four *P. citrophthora* strains. A value < 1 mg/mL was also selected as a threshold for the MIC. Seven extracts of *A. afra* (MIC 0.547 mg/mL), *O. europaea* (MIC 0.703 mg/mL), *D. rotundifolia* (MIC 0.860 mg/mL), *E. regia* (MIC 0.469 mg/mL), *M. longifolia* (MIC 0.938 mg/mL), *P. africanum* (MIC 0.938 mg/mL) and *C. sativa* (MIC 0.781 mg/mL) showed high inhibition against *P. citrophthora*, with all MIC values < 1 mg/mL and these seven extracts were fractionated for further investigation.

3.4 | *UPLC–QTOF-MS* Analysis of Artemisia Afra, *Olea europaea* and Peltophorum Africanum for Tentative Bioactive Identification

The bioassays of each of the seven fractions of the selected seven species are shown in Table 2. The results revealed that the highest activity against both pathogens for all plants was mainly in fractions f3, f4 and f5 based on their MIC values ≤ 0.625 mg/mL. This indicated that the major components are present in medium polar (fractions 3 and 4) and least polar (fraction 5) solutions of acetonitrile, methanol and water. The lowest MIC (0.156 mg/mL) was obtained in fraction f5 from *A. afra* against both pathogens.

To identify the possible bioactive compounds in the most active fractions, a UPLC–QTOF-MS profile was obtained for each of the fractions. The UPLC MS positive mode ionisation for fraction 5, *Artemisia afra*, is shown in Figure 1a. The major ionisation peak was detected at 7.00 min and was tentatively identified as scopoletin (Table 3). Similarly, the UPLC–QTOF-MS analysis of negative mode ionisation of fraction 4, *Olea europaea*, is shown in Figure 1b. The most prominent peak at a retention time of 7.48 min was selected for identification. Based on the mass spectral analysis and formula, the compound was tentatively identified as oleuropeinic acid (Table 3).

Figure 1c shows the positive mode ionisation of fraction 3, *P. africanum*, as determined by UPLC–QTOF-MS analysis. At a retention time of 6.29 min, the most intense peak was chosen for identification. The compound was tentatively identified as bergenin based on the mass spectral analysis and formula (Table 3).

3.5 | In Vitro Effect of DCM/MeOH of the Seven Most Potent Extracts on Mycelial Growth

The evaluation of DCM/MeOH extracts of the seven most active plant extracts on mycelium growth of *P. nicotianae* and *P. citrophthora* revealed significant antimicrobial activity ($p < 0.05$) at the two tested concentrations (Figure 2a,b). The fungicide, azoxystrobin (6.0 mg/mL), exhibited the highest level of inhibition (99.20%). All seven DCM/MeOH extracts exhibited > 60% inhibition of mycelial growth at the highest test concentration for both pathogens. In Figure 2a, at 2.5 mg/mL, the DCM/MeOH extracts from *A. afra*, *O. europaea* and *P. africanum* had the highest mycelium growth inhibition with values of 93.61%, 80.33% and 89.51%, respectively, while at a lower concentration of 1.25 mg/mL, a significant inhibition effect was also observed as all seven plants exhibited > 40% inhibition.

At a concentration of 2.5 mg/mL, significant mycelium growth inhibition was displayed by *A. afra*, *O. europaea* and *P. africanum* extracts on *P. citrophthora*, with inhibition values of 97.4%, 87.9% and 78.2%, respectively. Lower values were obtained at a concentration of 1.25 mg/mL, while the highest mycelium growth inhibition was exhibited by *A. afra* with 76.30% inhibition (Figure 2b).

Extracts from *A. afra* showed > 90% inhibition at 2.5 mg/mL for both pathogens, which is similar to the values (99%) obtained for the positive control. Specifically, at both concentrations, the efficacy of *A. afra* extracts against the two tested pathogens was higher than that of other tested extracts.

3.6 | In Vitro Effect of the Ethanol Extract of Artemisia Afra at Different Test Concentrations on Phytophthora Mycelial Growth

The results shown in Figure 3a,b demonstrate that the ethanol extract of *A. afra* inhibited the mycelium growth of both *Phytophthora* species (*P. nicotianae*: P. 121, P. 137, P. 1459 and

TABLE 1 | Minimum inhibitory concentration (MIC) mg/mL of extracts of 31 plant species and azoxystrobin against (a) *P. nicotianae* and (b) *P. citrophthora*.

(a)

	Phytophthora nicotianae				
	P. 1484	**P. 121**	**P. 1459**	**P. 137**	**(MIC) mg/mL**
Plant name	**MIC mg/mL**	**MIC mg/mL**	**MIC mg/mL**	**MIC mg/mL**	**Mean ± SD**
Olea europaea	0.625 ± 0.080^{a}	1.250 ± 0.301^{b}	1.25 ± 0.352^{c}	0.313 ± 0.031^{b}	0.800 ± 0.201^{b}
Foeniculum vulgare	1.250 ± 0.210^{c}	0.625 ± 0.030^{c}	0.313 ± 0.021^{b}	2.500 ± 0.521^{d}	1.172 ± 0.381^{c}
Artemisia afra	0.156 ± 0.001^{a}	0.313 ± 0.103^{b}	0.313 ± 0.021^{b}	0.313 ± 0.025^{c}	0.274 ± 0.080^{a}
Mentha longifolia	0.313 ± 0.120^{b}	1.250 ± 0.301^{c}	0.625 ± 0.068^{a}	0.625 ± 0.120^{b}	0.703 ± 0.340^{b}
Gymnosporia senegalensis	1.250 ± 0.580^{b}	1.250 ± 0.301^{c}	1.250 ± 0.130^{bc}	1.250 ± 0.456^{c}	1.250 ± 0.580^{d}
Dombeya rotundifolia	0.625 ± 0.280^{a}	0.156 ± 0.000^{a}	0.313 ± 0.120^{c}	2.500 ± 0.580^{d}	0.896 ± 0.240^{c}
Maytenus procumbens	0.625 ± 0.351^{b}	0.625 ± 0.040^{a}	0.313 ± 0.120^{b}	2.500 ± 0.580^{d}	1.016 ± 0.466^{c}
Vangueria infausta	NA	1.250 ± 0.301^{c}	2.500 ± 0.582^{d}	0.313 ± 0.150^{c}	1.354 ± 0.496^{c}
Opuntia ficus indica	1.250 ± 0.130^{bc}	0.313 ± 0.120^{b}	0.625 ± 0.106^{b}	1.250 ± 0.520^{d}	0.860 ± 0.406^{b}
Cassia beareana	2.500 ± 0.580^{d}	2.500 ± 0.580^{c}	NA	NA	2.500 ± 0.580^{cd}
Felicia muricata muricata	0.313 ± 0.120^{a}	0.313 ± 0.151^{b}	1.250 ± 0.520^{d}	2.500 ± 0.580^{d}	1.094 ± 0.497^{c}
Ziziphus mucronata	0.625 ± 0.108^{b}	1.250 ± 0.204^{b}	1.250 ± 0.520^{d}	NA	1.042 ± 0.395^{d}
Eucomis regia	0.313 ± 0.058^{a}	1.250 ± 0.200^{d}	0.625 ± 0.140^{b}	1.250 ± 0.258^{c}	0.860 ± 0.271^{b}
Peltophorum africanum	0.625 ± 0.130^{ab}	0.625 ± 0.070^{b}	0.313 ± 0.134^{a}	0.625 ± 0.131^{b}	0.547 ± 0.135^{ab}
Trichilia emetica	1.250 ± 0.288^{d}	0.625 ± 0.070^{bc}	2.500 ± 0.810^{d}	0.625 ± 0.310^{b}	1.250 ± 0.509^{bc}
Diospyros natalensis	0.625 ± 0.125^{b}	1.250 ± 0.310^{cd}	1.250 ± 0.013^{d}	0.625 ± 0.310^{a}	0.938 ± 0.313^{b}
Sonchus wilmsii	1.250 ± 0.508^{d}	NA	0.313 ± 0.021^{a}	NA	0.782 ± 0.469^{b}
Eugenia zeheri	1.250 ± 0.520^{d}	$2.500 \pm 0{,}648^{d}$	1.250 ± 0.358^{c}	1.250 ± 0.512^{b}	1.563 ± 0.541^{bc}
Scilla natalensis	2.500 ± 0.640^{a}	$1.250 \pm 0{,}079^{d}$	0.625 ± 0.225^{b}	2.500 ± 0.580^{b}	1.719 ± 0.812^{a}
Salvia triangularis	1.250 ± 0.580^{b}	$1.250 \pm 0{,}038^{d}$	1.250 ± 0.356^{d}	1.250 ± 0.506^{d}	1.250 ± 0.400^{c}
Ricinus communis	2.500 ± 0.640^{a}	$2.500 \pm 0{,}548^{b}$	1.250 ± 0.415^{a}	$2.500 \pm 0{,}548^{b}$	2.189 ± 0.541^{bc}
Cannabis sativa	0.625 ± 0.580^{a}	0.625 ± 0.256^{c}	0.313 ± 0.062^{b}	0.625 ± 0.062^{b}	0.547 ± 0.166^{a}
Citrus sinensis	0.313 ± 0.100^{b}	1.250 ± 0.129^{d}	2.500 ± 0.512^{c}	0.313 ± 0.017^{a}	1.094 ± 0.497^{b}
Ximenia caffra caffra	1.250 ± 0.125^{d}	0.313 ± 0.200^{b}	0.625 ± 0.580^{a}	1.250 ± 0.125^{cb}	0.860 ± 0.206^{b}
Sideroxylon inerme	2.500 ± 0.512^{c}	2.500 ± 0.580^{b}	NA	1.250 ± 0.120^{c}	2.083 ± 0.589^{bc}
Datura stramonium	0.313 ± 0.06^{ab}	NA	NA	0.625 ± 0.290^{d}	0.469 ± 0.156^{a}
Aloe tenuior	0.625 ± 0.105^{c}	1.250 ± 0.520^{d}	1.250 ± 0.306^{d}	0.625 ± 0.230^{b}	0.937 ± 0.313^{b}
Plumeria rubra	1.250 ± 0.310^{bc}	1.250 ± 0.405^{c}	1.250 ± 0.250^{d}	1.250 ± 0.230^{b}	1.250 ± 0.582^{a}
Euclea crispa	0.625 ± 0.175^{a}	0.625 ± 0.150^{b}	NA	2.500 ± 0.580^{c}	1.250 ± 0.384^{c}
Asparagus aethiopicus	1.250 ± 0.006^{d}	0.625 ± 0.150^{b}	0.313 ± 0.404^{a}	0.625 ± 0.034^{c}	0.703 ± 0.140^{b}
Psidium guajava	0.313 ± 0.120^{b}	0.313 ± 0.125^{a}	1.250 ± 0.606^{a}	1.250 ± 0.308^{bc}	0.781 ± 0.469^{b}
Azoxystrobin	0.625 ± 0.230^{b}	0.625 ± 0.170^{a}	0.156 ± 0.001^{a}	0.625 ± 0.010^{a}	0.508 ± 0.203^{ab}

(b)

	Phytophthora citrophthora				
	P. 2476	**P. 1465**	**P. 2534**	**P. 1988**	**(MIC) mg/mL**
Plant name	**MIC mg/mL**	**MIC mg/mL**	**MIC mg/mL**	**MIC mg/mL**	**Mean ± SD**
Olea europaea	0.625 ± 0.050^{ab}	1.250 ± 0.201^{b}	0.625 ± 0.252^{c}	0.313 ± 0.031^{b}	0.703 ± 0.093^{a}

(Continues)

TABLE 1 | (Continued)

(b)

	Phytophthora citrophthora				
	P. 2476	P. 1465	P. 2534	P. 1988	(MIC) mg/mL
Plant name	**MIC mg/mL**	**MIC mg/mL**	**MIC mg/mL**	**MIC mg/mL**	**Mean ± SD**
Foeniculum vulgare	1.250 ± 0.410^{b}	1.250 ± 0.030^{d}	1.250 ± 0.301^{b}	0.313 ± 0.031^{b}	1.016 ± 0.269^{c}
Artemisia afra	0.313 ± 0.002^{a}	0.625 ± 0.030^{b}	0.625 ± 0.121^{b}	0.625 ± 0.003^{a}	0.547 ± 0.056^{a}
Mentha longifolia	1.250 ± 0.210^{c}	1.250 ± 0.030^{c}	0.625 ± 0.021^{b}	0.625 ± 0.201^{a}	0.938 ± 0.261^{b}
Gymnosporia senegalensis	1.250 ± 0.120^{b}	0.313 ± 0.051^{a}	0.625 ± 0.068^{a}	1.250 ± 0.220^{b}	0.860 ± 0.169^{bc}
Dombeya rotundifolia	0.625 ± 0.580^{b}	1.250 ± 0.301^{c}	1.250 ± 0.301^{c}	0.313 ± 0.456	0.860 ± 0.107^{b}
Maytenus procumbens	2.500 ± 0.580^{d}	2.500 ± 0.580^{d}	0.625 ± 0.120^{c}	1.250 ± 0.301^{c}	1.719 ± 0.938^{d}
Vangueria infausta	NA	2.500 ± 0.380^{d}	0.625 ± 0.280^{b}	0.625 ± 0.180^{b}	1.250 ± 0.383^{c}
Opuntia ficus indica	2.500 ± 0.580^{d}	1.250 ± 0.130^{bc}	0.625 ± 0.140^{a}	0.625 ± 0.040^{b}	1.250 ± 0.484^{c}
Cassia beareana	NA	NA	0.625 ± 0.040^{c}	0.313 ± 0.120^{b}	0.469 ± 0.021^{a}
Felicia muricata muricata	NA	2.500 ± 0.580^{d}	0.313 ± 0.120^{b}	NA	1.407 ± 0.546^{d}
Ziziphus mucronata	2.500 ± 0.580^{d}	NA	0.625 ± 0.106^{b}	1.250 ± 0.130^{c}	1.458 ± 0.655^{d}
Eucomis regia	0.625 ± 0.108^{b}	0.625 ± 0.108^{ab}	0.313 ± 0.108^{b}	0.313 ± 0.151^{a}	0.469 ± 0.180^{a}
Peltophorum africanum	0.625 ± 0.130^{ab}	1.250 ± 0.204^{b}	1.250 ± 0.204^{b}	0.625 ± 0.070^{b}	0.938 ± 0.381^{b}
Trichilia emetica	1.250 ± 0.204^{b}	1.250 ± 0.204^{b}	0.625 ± 0.520^{d}	0.625 ± 0.520^{d}	0.938 ± 0.280^{b}
Diospyros natalensis	2.500 ± 0.640^{a}	1.250 ± 0.508^{d}	0.625 ± 0.520^{d}	0.313 ± 0.151^{b}	1.172 ± 0.567^{d}
Sonchus wilmsii	NA	1.250 ± 0.508^{d}	1.250 ± 0.310^{cd}	2.500 ± 0.810^{d}	1.667 ± 0.722^{cd}
Eugenia zeheri	1.250 ± 0.508^{cd}	2.500 ± 0.810^{a}	0.625 ± 0.125^{b}	0.625 ± 0.125^{bc}	1.250 ± 0.484^{d}
Scilla natalensis	NA	NA	0.625 ± 0.125^{a}	0.625 ± 0.125^{b}	0.625 ± 0.100^{a}
Salvia triangularis	1.250 ± 0.288^{d}	2.500 ± 0.640^{a}	0.625 ± 0.125^{bc}	1.250 ± 0.158^{c}	1.406 ± 0.586^{cd}
Ricinus communis	2.500 ± 0.640^{a}	1.250 ± 0.210^{b}	1.250 ± 0.310^{c}	2.500 ± 0.520^{d}	1.875 ± 0.721^{d}
Cannabis sativa	0.625 ± 0.125^{c}	0.625 ± 0.125^{b}	0.625 ± 0.105^{a}	1.250 ± 0.013^{d}	0.781 ± 0.313^{b}
Citrus sinensis	2.500 ± 0.640^{a}	2.500 ± 0.640^{a}	0.625 ± 0.125^{b}	0.625 ± 0.105^{bc}	1.563 ± 0.583^{d}
Ximenia caffra caffra	1.250 ± 0.129^{d}	$2.500 \pm 0{,}548^{d}$	1.250 ± 0.415^{a}	1.250 ± 0.215^{b}	1.563 ± 0.625^{c}
Sideroxylon inerme	NA	NA	0.625 ± 0.580^{a}	0.313 ± 0.100^{b}	0.469 ± 0.101^{a}
Datura stramonium	NA	NA	2.500 ± 0.512^{b}	2.500 ± 0.512^{c}	2.500 ± 0.300^{bc}
Aloe tenuior	1.250 ± 0.125^{d}	0.625 ± 0.256^{c}	0.625 ± 0.225^{b}	2.500 ± 0.640^{a}	1.250 ± 0.884^{d}
Plumeria rubra	1.250 ± 0.125^{d}	1.250 ± 0.125^{d}	$2.500 \pm 0{,}548^{b}$	1.250 ± 0.125^{d}	1.563 ± 0.525^{c}
Euclea crispa	NA	NA	$1.250 \pm 0{,}079^{d}$	0.625 ± 0.256^{c}	0.938 ± 0.442^{b}
Asparagus aethiopicus	2.500 ± 0.640^{a}	2.500 ± 0.248^{b}	0.625 ± 0.125^{ab}	0.625 ± 0.225^{b}	1.563 ± 0.183^{e}
Psidium guajava	1.250 ± 0.125^{d}	2.500 ± 0.512^{c}	1.250 ± 0.520^{d}	$1.250 \pm 0{,}038^{d}$	1.563 ± 0.625^{b}
Azoxystrobin	0.625 ± 0.125^{a}	0.313 ± 0.06^{ab}	0.313 ± 0.100^{a}	0.625 ± 0.056^{bc}	0.469 ± 0.080^{a}

Note: The values are the mean of three independent replicates, and the Mean values followed by different superscripts (small letters) are significantly different according to Tukey's test ($p < 0.05$).
Abbreviation: NA, not active.

P. citrophthora: P. 2534, P. 1465, P. 1988) compared to the positive control. At the highest tested concentration of 5 mg/mL, *A. afra* extract significantly inhibited *P. nicotianae* mycelium growth with 91.36%, 94.49% and 92.39% inhibition, which was higher than the values obtained for azoxystrobin fungicide (6.0 mg/mL), 88.92%, 90.38% and 88.02% (Figure 3a). Similarly, *A. afra* inhibited *P. citrophthora* mycelium growth with 84.81%, 82.53% and 80.98% inhibition compared to the azoxystrobin fungicide (6.0 mg/mL) 88.92%, 90.38% and 88.02% (Figure 3b).

The results from the amended media assay revealed that all concentrations of the tested plant extracts exhibited a significant ($p < 0.05$) inhibition of mycelium growth (Figure 4a,b). Most noticeably, the inhibitory effect of *A. afra* extract at 5 mg/mL

TABLE 2 | Minimum inhibitory concentration (MIC) mg/mL of 49 SPE fractions against *Phytophthora nicotianae* and *Phytophthora citrophthora*.

	Anti-*Phytophthora* activities of fractions 1–7						
	P. nicotianae				**MIC ± SD (mg/mL)**		
Plant species (family)	**f1**	**f2**	**f3**	**f4**	**f5**	**f6**	**f7**
Mentha longifolia (Lamiaceae)	1.25 ± 0.02	2.5 ± 0.03	0.625 ± 0.02	0.625 ± 0.02	0.313 ± 0.01	2.5 ± 0.03	1.25 ± 0.02
Olea europea (Oleaceae)	1.25 ± 0.02	1.25 ± 0.02	0.625 ± 0.02	0.313 ± 0.01	0.625 ± 0.02	1.25 ± 0.02	1.25 ± 0.02
Eucomia regia (Asparagaceae)	1.25 ± 0.02	2.5 ± 0.03	0.625 ± 0.02	0.625 ± 0.02	0.625 ± 0.00	0.625 ± 0.00	0.625 ± 0.01
Peltophorum africanum (Fabaceae)	2.5 ± 0.02	0.625 ± 0.01	0.313 ± 0.01	0.313 ± 0.02	1.25 ± 0.02	1.25 ± 0.02	1.25 ± 0.02
Cannabis sativa (Cannabaceae)	0.625 ± 0.00	1.25 ± 0.02	0.313 ± 0.01	0.625 ± 0.02	0.313 ± 0.02	0.625 ± 0.01	1.25 ± 0.02
Dombeya rotundifolia (Malvaceae)	2.5 ± 0.02	1.25 ± 0.01	1.25 ± 0.02	0.313 ± 0.01	0.625 ± 0.02	1.25 ± 0.02	1.25 ± 0.01
Artemisia afra (Asteraceae)	0.625 ± 0.01	1.25 ± 0.02	0.313 ± 0.00	0.313 ± 0.00	0.156 ± 0.00	0.313 ± 0.01	0.625 ± 0.01
	P. citrophthora				**MIC ± SD (mg/mL)**		
	f1	**f2**	**f3**	**f4**	**f5**	**f6**	**f7**
Mentha longifolia (Lamiaceae)	2.5 ± 0.03	2.5 ± 0.03	0.625 ± 0.02	0.625 ± 0.02	1.25 ± 0.02	2.5 ± 0.03	2.5 ± 0.02
Olea europea (Oleaceae)	0.625 ± 0.02	2.5 ± 0.03	1.25 ± 0.02	0.313 ± 0.00	0.625 ± 0.01	0.625 ± 0.01	1.25 ± 0.02
Eucomia regia (Asparagaceae)	1.25 ± 0.01	2.5 ± 0.04	0.313 ± 0.01	0.313 ± 0.00	0.625 ± 0.02	1.25 ± 0.02	2.5 ± 0.02
Peltophorum africanum (Fabaceae)	0.625 ± 0.01	1.25 ± 0.02	0.313 ± 0.00	0.625 ± 0.01	0.313 ± 0.01	1.25 ± 0.02	0.625 ± 0.00
Cannabis sativa (Cannabaceae)	1.25 ± 0.01	2.5 ± 0.02	0.625 ± 0.00	0.625 ± 0.00	0.625 ± 0.00	1.25 ± 0.01	1.25 ± 0.01
Dombeya rotundifolia (Malvaceae)	1.25 ± 0.01	2.5 ± 0.02	0.625 ± 0.00	0.625 ± 0.00	0.313 ± 0.00	1.25 ± 0.01	0.625 ± 0.01
Artemisia afra (Asteraceae)	1.25 ± 0.01	0.625 ± 0.00	0.625 ± 0.01	0.313 ± 0.00	0.156 ± 0.00	0.313 ± 0.01	1.25 ± 0.02

Note: The results are the mean of three replicates; Green represents the bio-active fractions.
Abbreviation: SD, standard deviation.

was more significant than the standard fungicide, azoxystrobin (6.0 mg/mL). Additionally, *A. afra* extracts were more effective against *P. nicotianae* than *P. citrophthora*.

3.7 | In Vivo Effects of the Ethanol Extract of *A. afra* on Citrus Brown Rot

The *A. afra* ethanol extract was tested at three concentrations (1.25 mg/mL, 2.5 mg/mL and 5 mg/mL) in in vivo trials against *P. nicotianae* and *P. citrophthora* using lemon fruit. The images in Figure 5a,b demonstrate the progression of brown rot lesions for each of the treatments. The results, presented in Figure 6a,b, revealed that the *A. afra* extract significantly ($p < 0.05$) reduced the severity of brown rot disease caused by both *Phytophthora* species. These treatments had a significant impact on *P. nicotianae* and *P. citrophthora*, according to the analysis of variance (Figure 6a,b). Every treatment was substantially different from the untreated controls. At 1.25 mg/mL, lemon fruit treated with the *A. afra* ethanol extract decreased brown rot severity from 97% in the untreated control to 72.30% and 88.83% for *P. nicotianae* (preventive and curative treatment), respectively. Similarly, disease severity was reduced from 98.42% to 79.65% and 82.71% in *P. citrophthora* preventive and curative treatments, respectively. At a concentration of 2.5 mg/mL, the *A. afra* ethanol extracts had a significant ($p < 0.05$) impact on the disease severity, with values slightly above 60% for curative and preventive treatments against both pathogens. However, at 5 mg/mL, *A. afra* extracts significantly ($p < 0.05$) reduced brown rot incidence to 47.20%

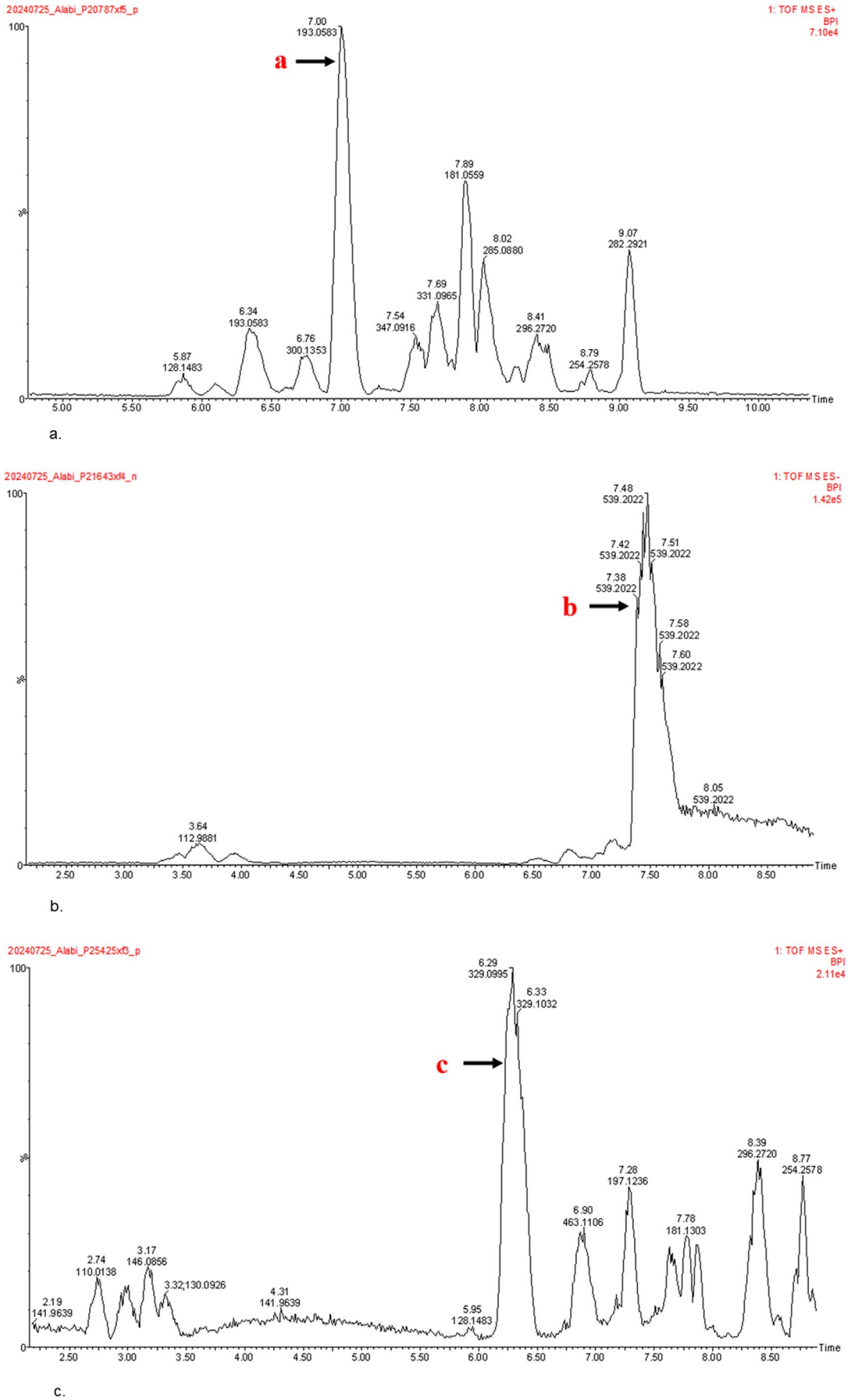
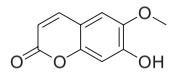
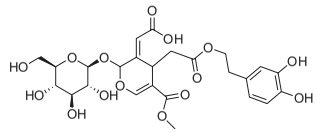
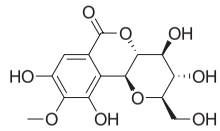


FIGURE 1 | (a) UPLC-HRMS analysis of *Artemisia afra* fraction 5 (f5) ESI positive mode. (b) UPLC-HRMS analysis of *Olea europaea* fraction 4 (f4), ESI negative mode. (c) UPLC-HRMS analysis of *Peltophorum africanum* fraction 3 (f3), ESI positive mode.

TABLE 3 | The tentative identification of selected peaks in *Artemisia afra*, *Olea europea* and *Peltophorum africanum* extracts.

Possible compound	Rt (min)	Acquired [M−H]—m/z	Adduct	Mass error (ppm)	Fit Conf. (%)	Possible formula of compound	Possible structure	Ref	Sample
Scopoletin	7.002	193.0486	$[M+H]^+$	−1.5	99.98	$C_{10}H_8O_4$		PubChem Lotus Knapsak Bora and Sharma (2011)	*Artemisia afra*
Oleuropeinic acid	7.747	539.1763	$[M-H]^-$	−1.1	NA	$C_{25}H_{30}O_{15}$		UNIFI Guinda et al. (2015)	*Olea europaea*
Bergenin	4.369	329.0865	$[M+H]^+$	−2.4	97.87	$C_{14}H_{16}O_9$		PubChem Lotus KEGG Bizimenyera et al. (2007)	*Peltophorum africanum*

Note: The compounds have been tentatively identified to a confidence level (Blaženović et al. 2018). Compound annotation outside the < 5 ppm mass error.
Abbreviations: Fit conf, fit confidence; m/z, mass to charge; ppm, parts per million; Rt, retention time.

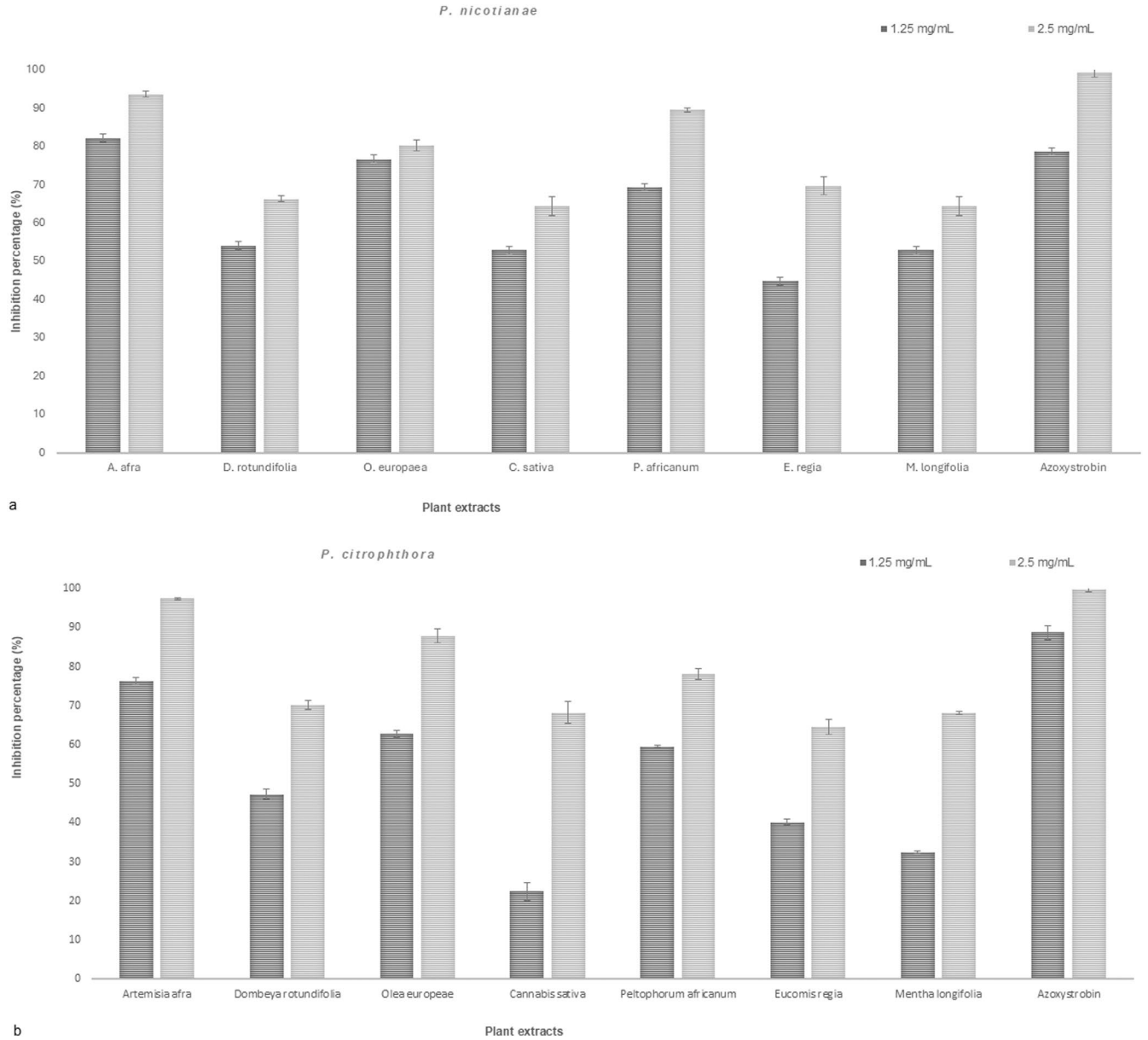


FIGURE 2 | (a) In vitro effect of seven active extracts at 1.25 mg/mL and 2.5 mg/mL concentrations on mycelia growth of *Phytophthora nicotianae*. Error bars indicate the standard error of the mean according to Tukey's HSD ($p < 0.05$). (b) In vitro effect of seven active extracts at 1.25 mg/mL and 2.5 mg/mL concentrations on mycelia growth of *Phytophthora citrophthora*. Error bars indicate the standard error of the mean according to Tukey's HSD ($p < 0.05$).

and 40.81% for *P. nicotianae*. Similarly, brown rot incidence was significantly ($p < 0.05$) reduced to 43.90% and 37.64% for *P. citrophthora* in the preventive and curative treatment, respectively.

4 | Discussion

Brown rot is a destructive disease which affects the yield and reduces the quality of citrus fruits by about 25%–30% (Xiao et al. 2023). *P. citrophthora* and *P. nicotianae* are the primary causative agents of citrus brown rot (Besoain et al. 1998; Mujica et al. 1980). Fungicides such as azoxystrobin and fludioxonil have been used to treat this pre and post-harvest disease (Merwe et al. 2023; Randall et al. 2014). However, these fungicides are costly and have numerous potential environmental and health side effects, such as skin and eye irritation, respiratory issues, endocrine disruption, neurological disorders and cancer (Sarkar et al. 2024). The development of resistant strains of *Phytophthora* spp. against fungicides is also a major concern. Recently, there has been a substantial increase in reports of *Phytophthora* species that are resistant to conventional fungicides (Kaur et al. 2025; Naqvi et al. 2024). Hence, there is a need to find an alternative to treat this disease (Chandra et al. 2022; Lu Fen et al. 2018).

Plant extracts, which contain various phytochemicals, including phenolic compounds, have garnered the attention of companies and scientists due to their antimicrobial properties (Álvarez-Martínez et al. 2020). Furthermore, phenolic compounds found

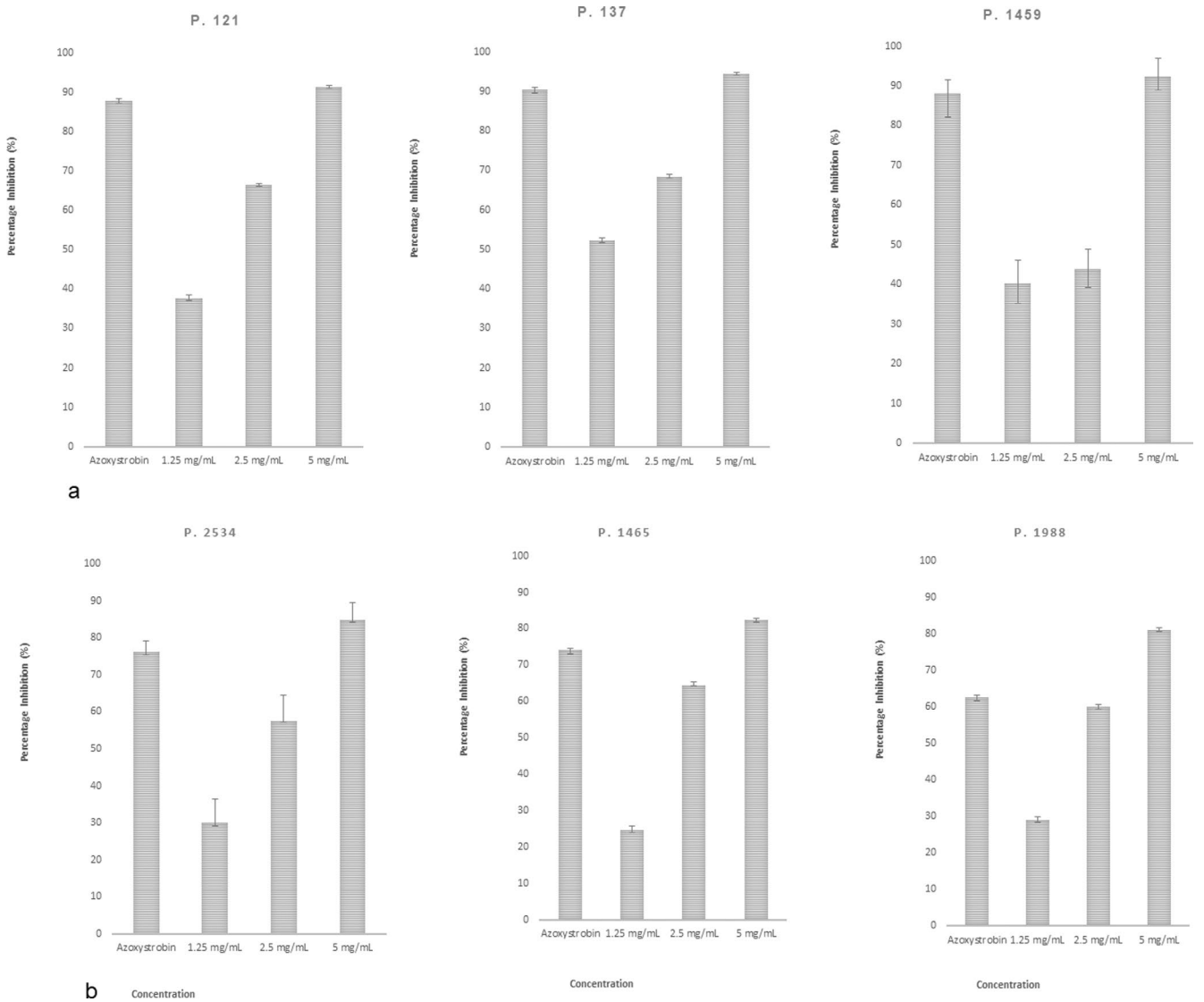


FIGURE 3 | (a) In vitro effect of azoxystrobin (6 mg/mL) and *Artemisia afra* ethanol extracts on mycelial growth of *P. nicotianae* (P. 121, P. 137 and P. 1459). Bars indicate the standard error of the mean according to Tukey's HSD ($p < 0.05$). (b) In vitro effect of azoxystrobin (6 mg/mL) and *Artemisia afra* ethanol extracts on mycelial growth of *P. citrophthora* (P. 2534, P. 1465 and P. 1988). Bars indicate the standard error of the mean according to Tukey's HSD ($p < 0.05$).

in plants are a significant source of biocides and preservatives that can be utilised as substitutes for synthetic fungicides to prevent fruit post-harvest diseases (Mastino et al. 2021).

DCM/MeOH extracts of 31 plants were studied for their efficacy in inhibiting the mycelium growth and zoospore germination of two *Phytophthora* spp. To date, none of these 31 plant extracts has been screened against the two *Phytophthora* species responsible for citrus brown rot. Most studies focused on investigating anti-fungal activities and fungal pathogens' susceptibility to medicinal plants (Zanna et al. 2021). However, screening against *Phytophthora* spp. has been very low (Bose et al. 2023; Di Ciaccio et al. 2020; El Khetabi et al. 2023; Liu et al. 2001).

Three concentrations of the DCM/MeOH extracts of 31 plant species were tested in vitro against *P. citrophthora* and *P. nicotianae*. The findings of the current study showed that the mycelium growth and zoospore germination of these species were significantly inhibited by extracts of *A. afra*, *O. europaea*, *P. africanum*, *D. rotundifolia*, *E. regia*, *C. sativa* and *M. longifolia* at all of the tested concentrations when compared to the positive control, and these plant extracts may thus hold potential for use in the prevention of post-harvest brown rot.

The results obtained after fractionation of the selected plant extracts clearly demonstrated the advantage of fractionation in improving biological activity and increasing the chance of isolating the bio-active compound in the plant extracts (Abubakar and Haque 2020). The activity of plant fractions (f3, f4 and f5), especially from *A. afra*, suggested that compounds of medium polarity were most likely responsible for the activity. This is similar to what was reported by Moyo et al. (2024), where *Artemisia annua* extract at $>200\,\mu g/mL$ was not potent against *Mycobacterium smegmatis*, but fraction f2 at $<50\,\mu g/mL$ was highly potent against this bacterium. In similar studies, an antimicrobial

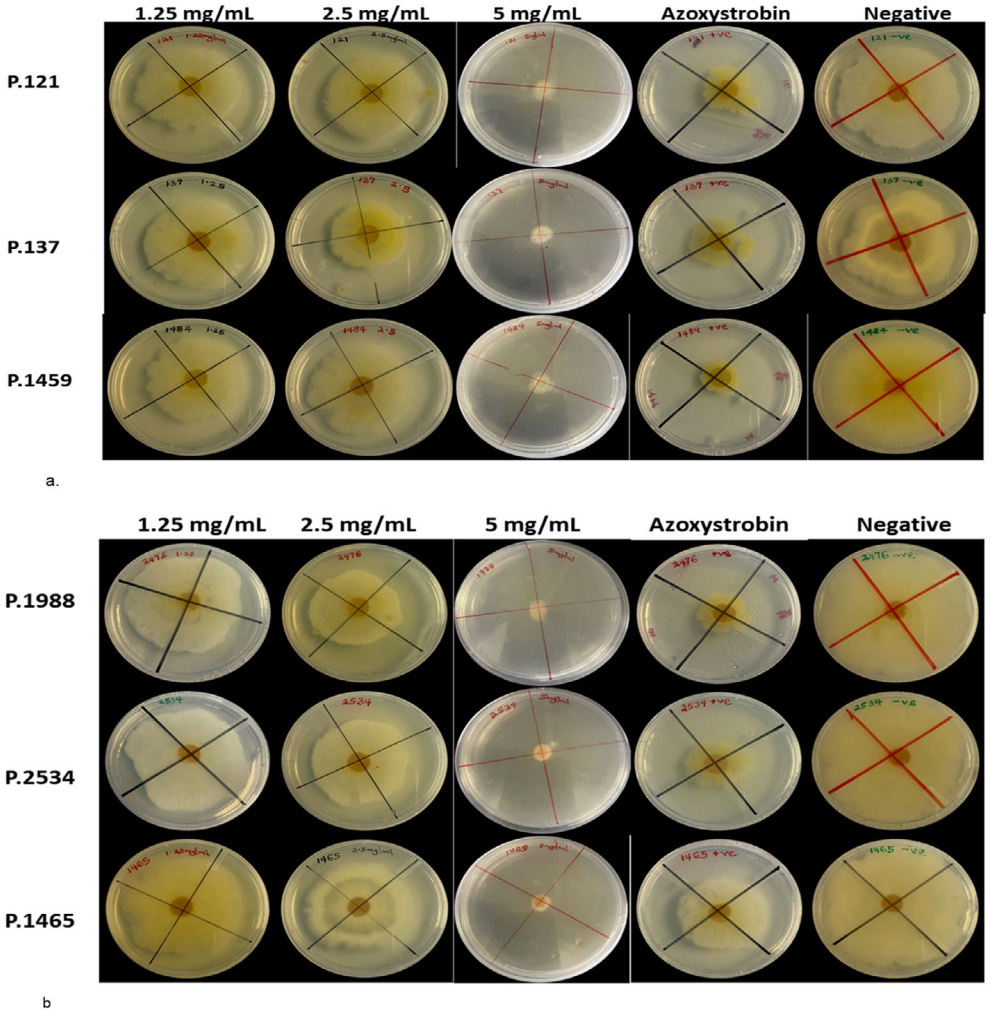


FIGURE 4 | (a) Effect of ethanol extracts of *Artemisia afra* on *Phytophthora nicotianae* growth in vitro. Representative figures of the *Phytophthora* growth on PDA medium supplemented with different concentrations of the plant extract, 1.25 mg/mL, 2.5 mg/mL and 5 mg/mL, with azoxystrobin (6.0 mg/mL) as a positive control and DMSO/SDW (1:39) in the medium as a negative control. (b) Effect of ethanol extracts of *Artemisia afra* on *Phytophthora citrophthora* growth in vitro. Representative figures of the *Phytophthora* growth on PDA medium supplemented with different concentrations of the plant extract, 1.25 mg/mL, 2.5 mg/mL and 5 mg/mL, with azoxystrobin (6.0 mg/mL) as a positive control and DMSO/SDW (1:39) in the medium as a negative control.

profiling of crude extracts and fractions of *Curatella americana* bark against *Candida albicans* revealed that significant inhibition was achieved by ethyl acetate fraction 2 with a MIC of 3.9–15.6 μg/mL, as compared to the crude extract, which was 15.3–31.3 μg/mL (de Mens Toledo et al. 2015). Furthermore, antifungal activity of *Ptaeroxylon obliquum* leaf extracts and fractions was tested against *Aspergillus niger*, and the results demonstrated a 146% increase in antifungal activity of the fraction as compared to the crude extract, indicating that fractionation can improve efficacy by concentrating active constituents and eliminating inhibitors (Ramadwa et al. 2024).

Amended medium assays revealed the inhibitory activity of DCM/MeOH extracts of *A. afra*, *O. europaea*, *P. africanum*, *D. rotundifolia*, *E. regia*, *C. sativa* and *M. longifolia*, with *A. afra* having the strongest mycelial growth inhibition (80%) of both pathogens, even at the lowest concentration of 1.25 mg/mL. These results were similar to those reported by Liu et al. (2001), who tested extracts from *Artemisia annua* against *Phytophthora capsici* at 1.25 mg/mL concentration. At 2.5 mg/mL, *A. afra*, *O. europaea* and *P. africanum* displayed strong inhibitory activity > 75% of mycelial growth of both *P. nicotianae* and *P. citrophthora*. Similarly, Rogozhin et al. (2020) reported that peptide extract from *Chelidonium majus*, *Inula helenium*, *Equisetum arvense*, *Laurus nobilis*, *Camellia sinensis* and *Hypericum perforatum*, at 2.0 mg/mL, significantly inhibit *P. infestans* mycelial growth. Additionally, other studies demonstrated that MeOH and ethyl acetate extracts of *Plectranthus barbatus*, *Tephrosia vogelii*, *Sphaeranthus suaveolens* and *Lantana camara* were effective against *P. infestans* at varying dosages. *Plectranthus barbatus* had the highest inhibition of mycelial growth, with 94% inhibition at a 30% (w/v) concentration (Ndala et al. 2019). In a similar study, Soumya and Nair (2012) examined the antifungal activity of aqueous extracts of *Capsicum frutescens* at a concentration of 10 mg/mL. The extracts demonstrated 88.06% and 88.33% inhibition, respectively, against *Aspergillus flavus* and *A. niger* species from citrus fruit. Our results are similar to those observed by Badillo et al. (2010), which revealed that *Artemisia ludoviciana* had the strongest effect at a concentration of 0.1 mg/mL, against *P. cactorum*, *P. capsici*, *P. cinnamomi*, *P. infestans*

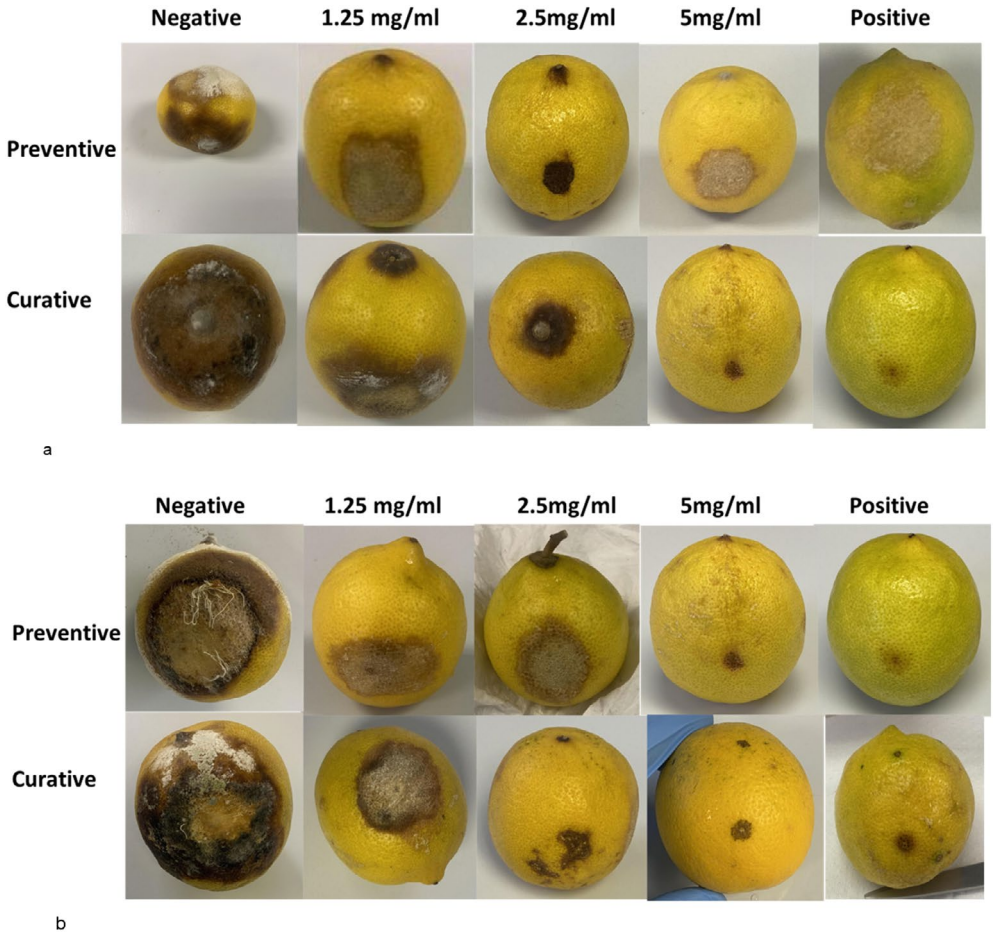


FIGURE 5 | (a) Brown rot lesions on lemon fruit treated with *A. afra* ethanol extracts and inoculated with *P. nicotianae* (negative control (DMSO/SDW 1:39), 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL and positive control azoxystrobin) for preventive and curative treatments. A zoospore concentration of 1×10^6 spores/mL was used, and the fruits were incubated for 7 days at 25°C. Negative control: Fruits treated with DMSO/SDW (1:39), positive control: Fruits treated with azoxystrobin at 6.0 mg/mL. (b) Brown rot lesions on lemon fruit treated with *A. afra* ethanol extracts and inoculated with *P. citrophthora* (negative control (DMSO/SDW 1:39), 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL and positive control azoxystrobin). A zoospore concentration of 1×10^6 spores/mL was used, and the fruits were incubated for 7 days at 25°C. Negative control: Fruits treated with DMSO/SDW (1:39), positive control: Fruits treated with azoxystrobin at 6.0 mg/mL.

and *P. mirabilis*, at rates of 100%, 100%, 100%, 60% and 100%, respectively. Additionally, a DMSO extract of *O. europaea* at a dosage of 5% (w/v) showed over 60% inhibition of mycelial growth of *P. infestans* (Río et al. 2003).

The inhibition of the mycelial growth of *P. nicotianae* and *P. citrophthora* by ethanol extracts might be due to the active constituents present in the plant and the interaction between the major and minor constituents. These phytoconstituents demonstrate strong anti-oomycete, antibacterial, antifungal, herbicidal, larvicidal, allelopathic and repellent properties (Bitchagno et al. 2022). These findings are consistent with several investigations that have linked the antibacterial properties of plant extracts, including various types of phenolic compounds, to the presence of polyphenols (Ndala et al. 2019; Rogozhin et al. 2020).

The phenolic components of plants (flavonoids, tannins and lignans) are the main chemicals that function as principal antioxidants or free radical terminators. Flavonoids are among the most diverse and widely distributed classes of natural compounds, making them important natural phenolics (Dambolena et al. 2010). UPLC-MS data analysis revealed that the three tentatively identified compounds (scopoletin, oleuropein and bergenin) are all phenolic compounds. A study has revealed that scopoletin, a coumarin with known ROS generation capacity, may disrupt the membrane integrity of oomycetes, cause oxidative damage and disturb essential cellular functions (Jacoby et al. 2021). In addition, phenolic-rich extracts have been shown to strongly inhibit cellulose synthase, disrupt ROS production and interfere with mitochondrial activities, leading to enzymatic hydrolysis of microbial cells (Chen et al. 2020; Hari et al. 2024). The effects of phenolic compounds can be both direct and indirect, reducing pathogen growth, improving plant defence, neutralising microbial systems, preventing spore germination, killing hyphae and enhancing cell permeability; therefore, phenolic components are typically very effective antimicrobial agents (El Khetabi et al. 2020; Joaquín-Ramos et al. 2020).

Although in vitro studies of plant extracts are an essential initial step in evaluating their antifungal potential against post-harvest pathogens, in vivo tests are required to ascertain whether the extracts are indeed effective (Tegegne et al. 2008). For the in vivo assay, an *A. afra* extract was chosen based on its

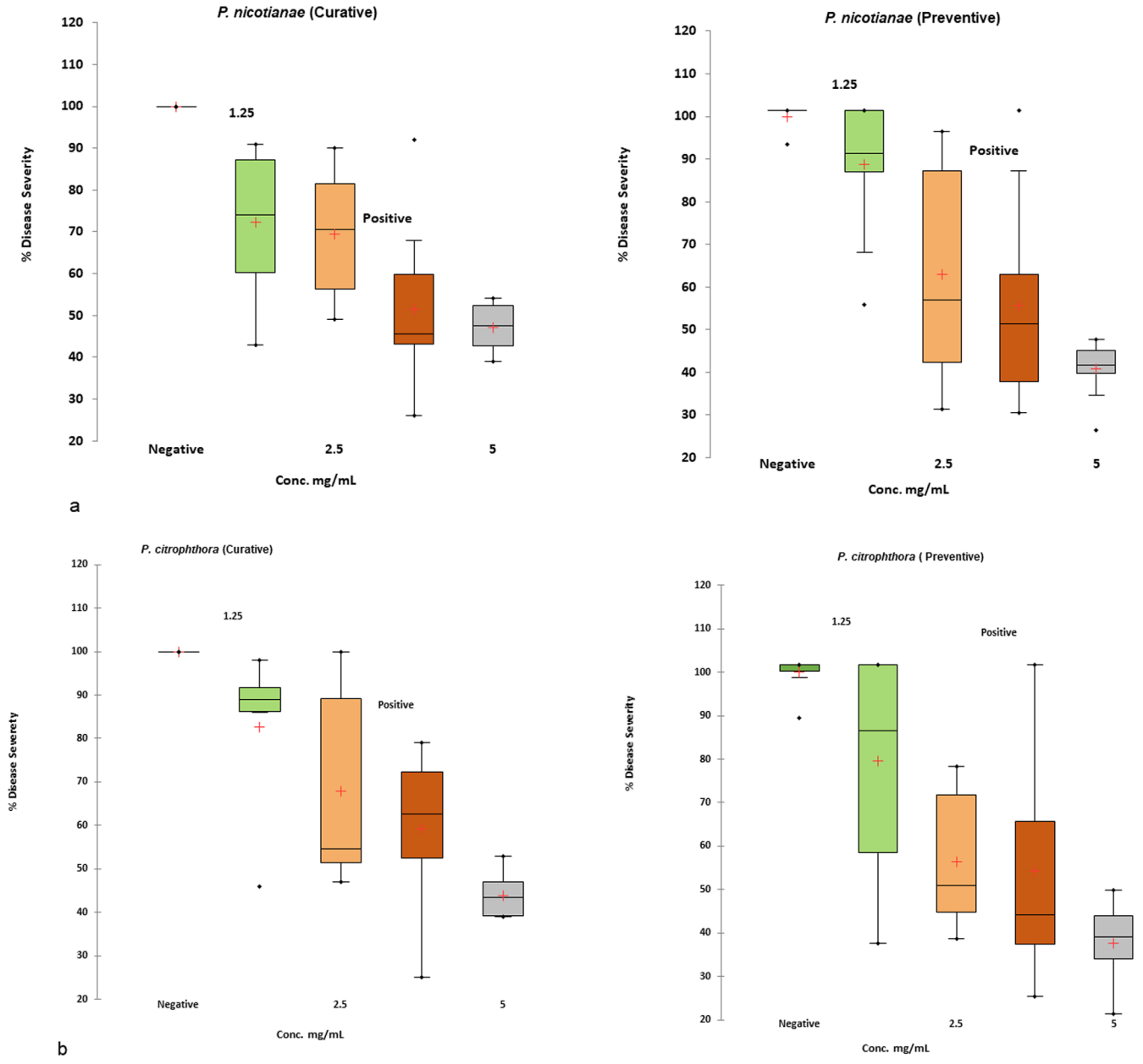


FIGURE 6 | (a) Effect of *A. afra* extracts on the disease severity of brown rot caused by *P. nicotianae* incubated at 25°C for 7 days. Significant ($p < 0.05$) differences between means obtained with each concentration are indicated with the error bars on each box according to Tukey's HSD ($p < 0.05$). (b) Effect of *A. afra* extracts on the disease severity of brown rot caused by *P. citrophthora* incubated at 25°C for 7 days. Significant ($p < 0.05$) differences between means obtained with each concentration are indicated with the error bars on each box according to Tukey's HSD ($p < 0.05$).

good performance in the in vitro assays. The extract tested at 1.25 mg/mL demonstrated a 20% decrease in disease severity as compared to the untreated fruits in both curative and preventive treatments. At the highest concentration of 5 mg/mL, the *A. afra* extract significantly decreased brown rot severity with superior performance to azoxystrobin in artificially wounded and inoculated lemon fruit. At all evaluated concentrations, the plant extracts showed no phytotoxic effects on fruit tissues. Curative and preventive treatments showed similar disease reduction patterns, as both treatments significantly decreased the disease severity at all dosage concentrations. Similarly, the effects of *Callisia fragrans* and *Azadirachta indica* aqueous extracts (6% w/v) were investigated against *Aspergillus niger* and *Colletrichum* species on citrus fruits. Both plant extracts reduced rot by 68.18% and 80.13% in an in vivo assay, respectively (Le et al. 2018). Ethanol extract of *Cerbera odollam* was tested against *P. digitatum*, at concentrations ranging from 500 to 5000 ppm. The results obtained showed that *C. odollam* increased the shelf life of citrus fruits above 3 weeks in storage and decreased the weight loss by 5.87% (Khaleel 2017).

The findings of this study demonstrated that plant extracts possess potent antimicrobial properties, which successfully reduced the severity of brown rot. This may be associated with the presence of phenolic constituents in the plant extracts, as they are an important source of natural agrochemicals, which are environmentally friendly and have less toxic effects on fruits than synthetic fungicides.

5 | Conclusion

This research showed that extracts from seven plant species were effective against both *P. nicotianae* and *P. citrophthora*. The findings of this study suggest that extracts from *A. afra*, *O. europaea* and *P. africanum* can be utilised as a safe and effective way to protect citrus fruits from infection by *Phytophthora* spp. Further research is required to isolate and purify the bioactive compounds tentatively identified, confirm their structure using NMR and retest to confirm the bioactivity thereof, before subsequent development into biological products for management of brown rot.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References denoted by an asterisk (*) are cited in Table S1.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Selected plant species evaluated for potential inhibition of *Phytophthora* species.