Research Article

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A comparative study of the antifungal efficacy and phytochemical composition of date palm leaflet extracts

https://doi.org/10.1515/chem-2024-0044
received April 8, 2024; accepted May 14, 2024

Abstract: This study rigorously evaluated the inhibitory effects of chloroform (PDCL) and methanol (PDML) extracts from date palm (*Phoenix dactylifera* L.) leaflets, in comparison to fosetyl-aluminum, against molecularly identified fungal strains *Fusarium oxysporum* (OR116511), *Botrytis cinerea* (OR116493), and *Rhizoctonia solani* (OR116530) isolated from date palm tree roots and fruits. We found that coumaric acid (1663.91 μg g^{-1}) is one of the top four high-performance liquid chromatography-major phenols in the PDML extract, while the PDCL extract includes rosmarinic acid (291.08 μg g^{-1}). The major flavonoids in the PDML extract are naringenin and kaempferol, whereas PDCL extract includes naringenin and quercetin. In the PDML extract, methyl 9-*cis*-11-*trans*-octadecadienoate (9.96%) is one of the top five gas chromatography–mass spectrometry major compounds; likewise, *cis*-13-octadecenoic acid (26.16%) is in the PDCL extract. The highest growth inhibition percentages of PDCL and PDML extracts were initiated against *F. oxysporum* (60.53 and 50.00%) at 150 μg mL^{-1}, respectively, whereas inhibition against *B. cinerea* was realized at the highest concentration with 50.82%. Fosetyl-Al potently inhibited the growth of fungal isolates to varying degrees. Therefore, we could successfully employ PDCL extract to control the growth of *F. oxysporum* and *B. cinerea* and also use both extracts against *R. solani*.

Keywords: date palm, *Rhizoctonia solani*, necrotrophic fungi, soilborne, root rot

1 Introduction

The increasing prevalence of drug-resistant pathogens and the adverse effects associated with conventional treatments underscore the urgent need for the identification of novel bioactive agents derived from medicinal plants. Despite their widespread availability, cost-effectiveness, and positive socio-cultural perceptions, it is noteworthy that the World Health Organization estimates that more than 80% of developing countries have yet to fully utilize traditional plant-based medicines. Nevertheless, plants are therapeutically useful owing to the abundance and novelty of the active phytochemicals compared to other sources [1,2]. The renaissance in phytochemistry technology gave rise to an outstanding usage of plant-origin bioactive substances or their synthetic equivalents [3].

Date palm tree, *Phoenix dactylifera* L. (Arecales: Arecaceae), assorted as angiosperms, monocotyledon, is deemed one of the native and traditional fruit tree crops in the tropics and semi-tropic provinces from the south of Asia or North Africa [4]. Its pinnate almost carries 150 leaflets. The leaflets have an approximate length of 30 cm and a thickness of 2 cm [5]. Assessment of the phytochemicals in the leaf extract of date palm, *P. dactylifera* L., revealed antioxidants, known by groups of tannin, alkaloid, terpenoid, carbohydrate, phenol, amino acid, and flavonoid. Since then, a growing trend has been

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developed to maximize the benefits of antioxidants of natural origin. Exceedingly, these compounds are worthy of further antifungal and human health considerations [6,7]. Even so, previous phytochemistry studies have not looked into the bioactive screening of date palm extracts against the growth activity of different oomycetes or necrotrophic fungi enough. Unless a prior study mentioned the inhibitory effects of acetone and methanolic leaf extracts of *P. dactylifera* var. *Barhee* and *Rothana* against the growth activity of the pathogenic fungus *Fusarium oxysporum* [8,9]. Fosetyl-aluminum (fosetyl-Al) is an organophosphorus compound belonging to the chemical class of phosphonates, which consists of an aluminum derivative of the phosphorous acid ethyl-ester (phosphite). Since fosetyl-Al was first invented in 1973 by Philagro, it has been registered as an acid-product fungicide. Its aluminum (Al^{3+}) ions lessen the pH in water solutions and act directly as antimicrobial agents against oomycete fungi [10,11]. Mbasa et al. [12] revealed that fosetyl-Al reduced incidences of wilt diseases in tomatoes caused by *F. oxysporum* under *in vivo* conditions. Furthermore, it has been known to have a complete inhibitory effect against *Botrytis cinerea* growth [13]. Different products of phosphite showed slight growth inhibition against *Rhizoctonia solani* [14].

Global instances of the well-known pathogens that threaten date palm yield are *R. solani*, *F. oxysporum*, and *B. cinerea* [15]. Date palm orchards are susceptible to varying degrees of root and fruit rot diseases, which are influenced by annual fluctuations in humidity and rainfall. Furthermore, Orole et al. [16] showed that date palm fruits harbor a substantial population of fungi, which leads to fruit rot, mycotoxin production, and a decrease in the economic value of date fruits. A variety of fungus species, including *Aspergillus niger*, *Penicillim* sp., *R. solani*, *Alternaria* sp., *Botryodiplodia theobromae*, *B. cinerea*, and *F. oxysporum*, were isolated from date roots and fruits and could lead to degradation of date palm [16–18]. The root diseases get more intricate when linked with other microbial infections, such as fungi that induce wilt and root rot in trees. During the management procedures, the farmers may neglect to consider the hazardous impact of the accompanying microflora, which can lead to a serious infection of root rot or wilt in the trees. As a result, the trees may be affected by a combination of diseases that may ultimately result in their weakened state or even their full demise. Several studies have indicated that pesticides have limited effectiveness in reducing the damage caused by date palm trees due to the complexity of the diseases involved [19–21]. In this regard, we examined the suppressive impact of leaflet extracts from *P. dactylifera* L. using chloroform (PDCL) and methanol (PDML) compared to fosetyl-Al on the growth of the fungal strains *B. cinerea*, *F. oxysporum*, and *R. solani*. The fungal isolates that were chosen were accurately identified by morphological and molecular methods. High-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) phytochemical screening of the tested extracts was performed in order to estimate their relevant bioactive components that may inhibit the fungal growth activity.

2 Materials and methods

2.1 Origin of fungal isolates

The present fungal strains were isolated from date palm roots and fruits and initially identified using a handbook for morphological identification [22]. A light microscope is utilized to identify fungal isolates at the genus level [22]. The molecular recognition of these isolates was achieved by amplifying the "Internal Transcribed Spacer" (ITS) region of the genes responsible for generating ribosomes using universal primers ITS1 and ITS4 [23]. The polymerase chain reactions (PCR) were previously documented by Behiry et al. [24]. The ultimate results of the PCR were subsequently elucidated, dispatched to the "Sanger sequencing procedure," and analyzed. The isolates were aligned using the GenBank website BLAST tool and then the identified sequences were subsequently submitted to the GenBank database to acquire their accession numbers.

2.2 Cold-extraction techniques of *P. dactylifera* L. leaflets

Leaflets of the date palm tree, *P. dactylifera*, were obtained from New Borg El Arab City, Egypt. *P. dactylifera* leaves were dried at 25°C for 2 weeks. A grinding mill (IKA MultiDrive basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) was used to produce a superfine powder from the dried leaves. Cold extraction of the leaflet powder was carried out using two solvents (96% purity): chloroform and methanol (Merck KGaA, Darmstadt, Germany). Over the course of a week, 100 g of dry powder was immersed in 500 mL of each solvent. The extracted solutions were then filtered and discarded using a rotary vacuum evaporator (IKA-Werke GmbH & Co. KG, Staufen, Germany). The crude extracts were stored in glass vials at a temperature of 4°C until laboratory studies were conducted. The crude extracts were produced in various quantities and eluted with dimethyl sulfoxide (DMSO).

2.3 Growth inhibition response to *P. dactylifera* leaflet extracts

The effectiveness of *P. dactylifera* leaflet extracts in inhibiting the growth activity of the specific isolates was evaluated using the radial growth test on poisoned food procedures [25]. Different concentrations of leaflet extracts were incorporated into the potato dextrose agar (PDA) plates to achieve final concentrations of 20, 50, 100, and 150 μg mL^{-1}. Fosetyl-aluminum (Fosetyl-Al, Aliette® 80% WP; Bayer AG, Leverkusen, Germany) is an organophosphorus fungicide compound that was utilized as a positive control at a concentration of 100 μg mL^{-1}, matching the recommendation of the "Agriculture Pesticides Committee" in Egypt.

The concentrations of each leaflet extract were compared to the negative control (DMSO-PDA) and the positive control (fosetyl-Al). The fungal strains were tested by placing circular discs (5 mm in diameter) on treated plates and incubating them at a temperature of 25°C for 5 days [25]. The experiment was replicated three times. The variations in radial growth diameters were quantified using inhibition percentages, calculated according to the formula established by Dissanayake [26] as follows:

$$\text{Growth inhibition (\%)} = [(P - D)/P] \times 100,$$

where P is the mycelial growth length (mm) in the negative control and D is the mycelial growth length (mm) of the tested leaflet extract (treatment) or fosetyl-Al (positive control).

2.4 HPLC screening

A total of 19 HPLC-standard chemicals were utilized to conduct phytochemical screening of the PDML and PDCL extracts. An Agilent 1260 series was used to analyze both of the crude extracts. The separation process was optimized using an Eclipse C18 column with dimensions of 4.6 mm × 250 mm i.d. and a particle size of 5 μm. The mobile phase, consisting of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B), was adjusted at a flow rate of 0.9 mL min^{-1}. A mobile phase linear gradient program was implemented with a step size of 1 min and durations of 5, 8, 12, 15, 16, and 20 min, using (A) concentrations of 82, 80, 60, 60, 82, 82, and 82%, respectively. A plethora of wavelength detectors were set at 280 nm. A 5 μL sample solution was administered. The column temperature was adjusted precisely to 40°C. The identified phytochemical components were compared to a set of 19 standard chemicals, serving as a reference index.

2.5 GC–MS screening

The leaflet extracts were analyzed using GC–MS with an Agilent 7000D instrument from Agilent Technologies in Santa Clara, CA, USA. The GC–MS model was fitted with a column consisting of a 5% diphenyl to 95% dimethylpolysiloxane mixture, as well as an HP-5MS capillary column. The carrier gas, consisting of 99.99% helium, was regulated to flow at a rate of 1 mL min^{-1}. The ionization energy scanning time was set at 70 eV at a rate of 0.2 s^{-1}. The fragment detection spanned from 40 to 600 m/z. Each 1 μL injection of the sample was split in a ratio of 10:1 at a temperature of 250°C. The oven temperatures of the column were initially set at 50°C with a rate of increase of 3 min^{-1}, then gradually increased by 10°C min^{-1} till reaching 280°C, and finally finished at 300°C with a rate of increase of 10 min^{-1}. The identified phytochemical components were compared to the genuine substances in the Wiley Registry 8E, Replib, and Mainlib libraries [27].

2.6 Statistical analysis

All data from laboratory testing were analyzed using a one-way analysis of variance. The Statistical Analysis System (SAS v.8.02, SAS Institute, Cary, NC, USA) [28] software significantly distinguished the means at the LSD 0.05 level.

3 Results

3.1 Characterization of fungal isolates

F. oxysporum, *R. solani*, and *B. cinerea* fungal isolates were consistently obtained from sections of infected tissues plated on PDA. The colonies of *F. oxysporum* exhibited a white color and showed micro and fusaria-shaped conidia under the light microscope. The *R. solani* isolate displayed septate hyphae branching at approximately 90°, while *B. cinerea* initially appeared white and later darkened to gray as spores differentiated. Furthermore, white sclerotia formed in the cultures, turning black after 3 days. The ITS region sequence results confirmed the initial identification of the fungal isolates. All isolates were deposited in GenBank under the names and accession numbers: *B. cinerea* isolate BC-108 (OR116493), *F. oxysporum* isolate FO-99 (OR116511), and *R. solani* isolate RHS-299 (OR116530). A phylogenetic tree was constructed to determine the related genera to our fungal isolates (Figure 1).

3.2 Growth response of fungal isolates to leaflet extracts of *P. dactylifera*

The results showed that the PDCL and PDML (Table 1) extracts at doses of 20, 50, 100, and 150 µg mL^{-1} significantly impacted on fungal growth compared to the negative and positive controls. The PDCL extract, at 150 µg mL^{-1}, was equivalent to fosetyl-Al but exhibited lower radial growth activity for *F. oxysporum* with a diameter of 15.00 mm with growth inhibition reached 60.53%. The PDCL extract at 150 µg mL^{-1} had the lowest growth activity for *B. cinerea* (20.33 mm). PDCL extract at 100 µg mL^{-1} (23.00 mm) and fosetyl-Al (25.00 mm) showed similar low growth rates for *B. cinerea*. All concentrations of PDCL extract showed limited growth activity against *R. solani*, similar to fosetyl-Al (25.00 mm). All concentrations of PDCL extract and fosetyl-Al dramatically reduced *R. solani* growth activity compared to the negative control.

All the concentrations of PDML extract and fosetyl-Al that were given greatly decreased the radial growth diameters of the tested fungal isolates compared to the negative control (Table 1). At a concentration of 150 µg mL^{-1}, the PDML extract had the same effectiveness as fosetyl-Al and had the best growth inhibitory efficacy against *F. oxysporum*, resulting in diameters of 21.00 and 16.67 mm. The PDML extract at 20 and 50 µg mL^{-1} had the lowest growth inhibition for *B. cinerea*. PDML extract (100 and 150 µg mL^{-1}) greatly slowed down the growth of *R. solani*, leading to diameters that were 21.33 and 19.33 mm compared to 25 mm for fosetyl-Al. Meanwhile, at all concentrations, PDML extract reduced the *R. solani* growth rate compared to the negative control (Table 1).

Overall, we were able to determine the inhibition percentages of the leaflet extracts that showed the highest level of inhibition against the fungal isolates (Table 1). The inhibition percentages of PDCL and PDML extracts at

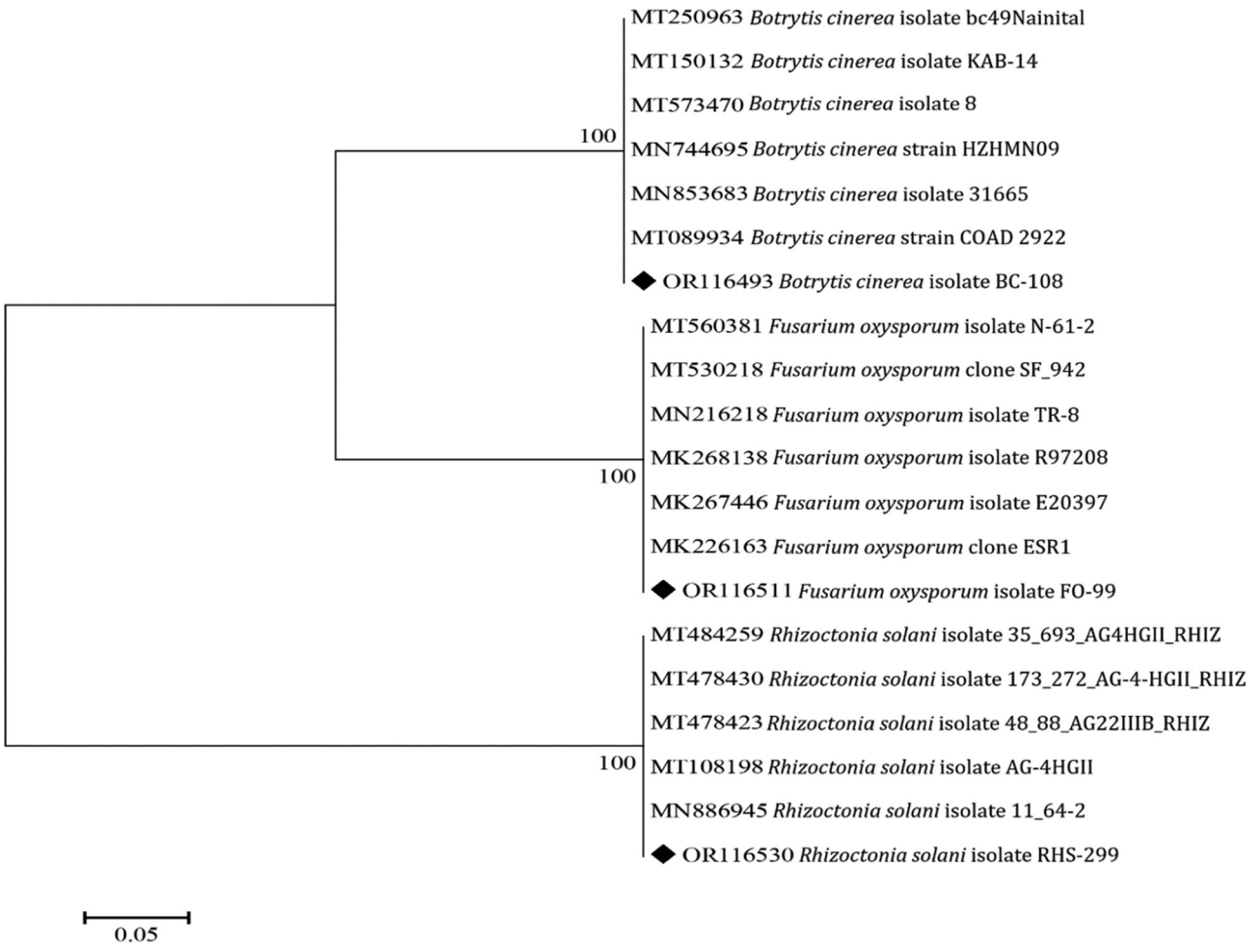

Figure 1: The phylogenetic tree based on the sequences of internal transcribed spacer gene sequences (ITS region) of the isolated fungal isolates *B. cinerea* isolate BC-108 (OR116493), *F. oxysporum* isolate FO-99 (OR116511), and *R. solani* isolate RHS-299 (OR116530), with related fungal isolates deposited in GenBank. The MEGA 11 software used the UPGMA algorithm and the bootstrapping method with 2,000 replicates to create the tree.

Table 1: Growth response of fungal isolates to series concentrations of chloroform and methanolic leaflets extracts of *P. dactylifera* compared to the fungicide (fosetyl-Al) after 5 days of incubation

	Concentrations (µg mL^{-1})	Growth [mean diameter (mm) ± SD[1]; inhibition (%)]					
		F. oxysporum		*B. cinerea*		*R. solani*	
PDCL	20	35.00^{ab} ± 0.52	7.89	27.00^{b} ± 0.17	33.61	27.33^{cde} ± 0.29	26.79
	50	31.33^{bc} ± 0.12	17.54	27.00^{b} ± 0.00	33.61	22.00^{b} ± 0.17	41.07
	100	31.00^{bc} ± 0.35	18.42	23.00^{cd} ± 0.00	43.44	21.00^{cde} ± 0.44	43.75
	150	15.00^{g} ± 0.00	60.53	20.33^{de} ± 0.06	50.00	20.67^{de} ± 0.40	44.64
PDML	20	30.33^{de} ± 0.21	20.18	24.33^{e} ± 0.12	40.16	25.67^{cde} ± 0.32	31.25
	50	26.67^{ef} ± 0.29	29.82	23.00c^{de} ± 0.17	43.44	23.33^{bc} ± 0.29	37.50
	100	25.33^{cd} ± 0.46	33.33	21.33^{cd} ± 0.06	47.54	21.33^{e} ± 0.12	42.86
	150	21.00^{bc} ± 0.17	44.74	20.00^{bc} ± 0.00	50.82	19.33^{bcde} ± 0.06	48.21
	−ve[2]	38.00^{a} ± 0.17	0.00	40.67^{a} ± 0.12	0.00	37.33^{a} ± 0.06	0.00
	+ve[3]	16.67^{fg} ± 0.06	56.14	25.00^{bc} ± 0.40	38.53	25.00^{bcd} ± 0.40	33.04

[1]Standard deviation. [2]Negative control (DMSO). [3]Positive control (fosetyl-Al fungicide,100 µg mL^{-1}). Similarity in the letters attached to the growth rates in each column does not significantly differ as per the $LSD_{0.05}$. *P. dactylifera* L. leaflet extracts with chloroform (PDCL) and methanol (PDML).

a concentration of 150 µg mL^{-1} inhibited the growth of *F. oxysporum* by 60.53 and 50%, respectively. The growth of *B. cinerea* was inhibited by the PDML extract at a concentration of 150 µg mL^{-1} with a value of 50.82%. The inhibition percentages of PDML and PDCL leaflet extracts, at a concentration of 150 µg mL^{-1}, demonstrated the greatest level of inhibition (48.21 and 44.64%, respectively) against *R. solani*.

3.3 HPLC analysis of *P. dactylifera* leaflets extracts

The HPLC analysis identified a total of 19 phytochemical components in the PDML and PDCL extracts, categorized into 12 phenolic and 7 flavonoid categories (Figures 2 and 3). The presence of phytochemical ingredients in both extracts was confirmed by analyzing their peak areas and concentrations

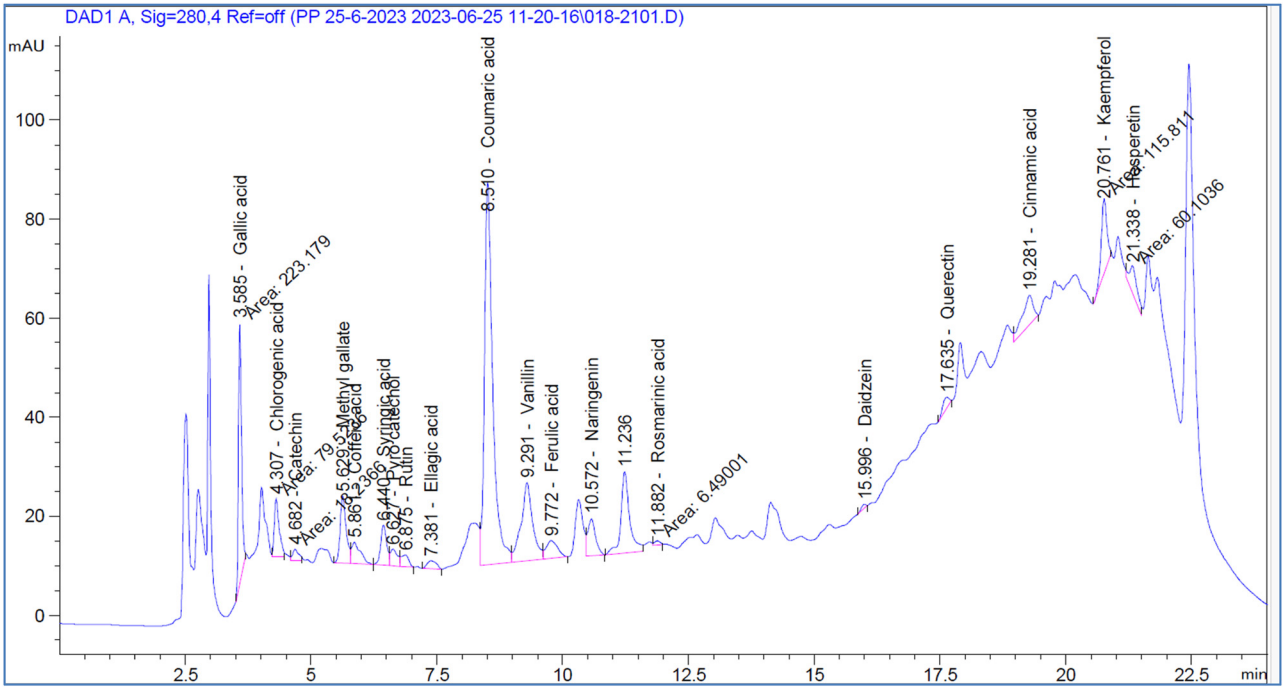

Figure 2: HPLC-phytochemical screening in *P. dactylifera* leaflets methanolic extract.

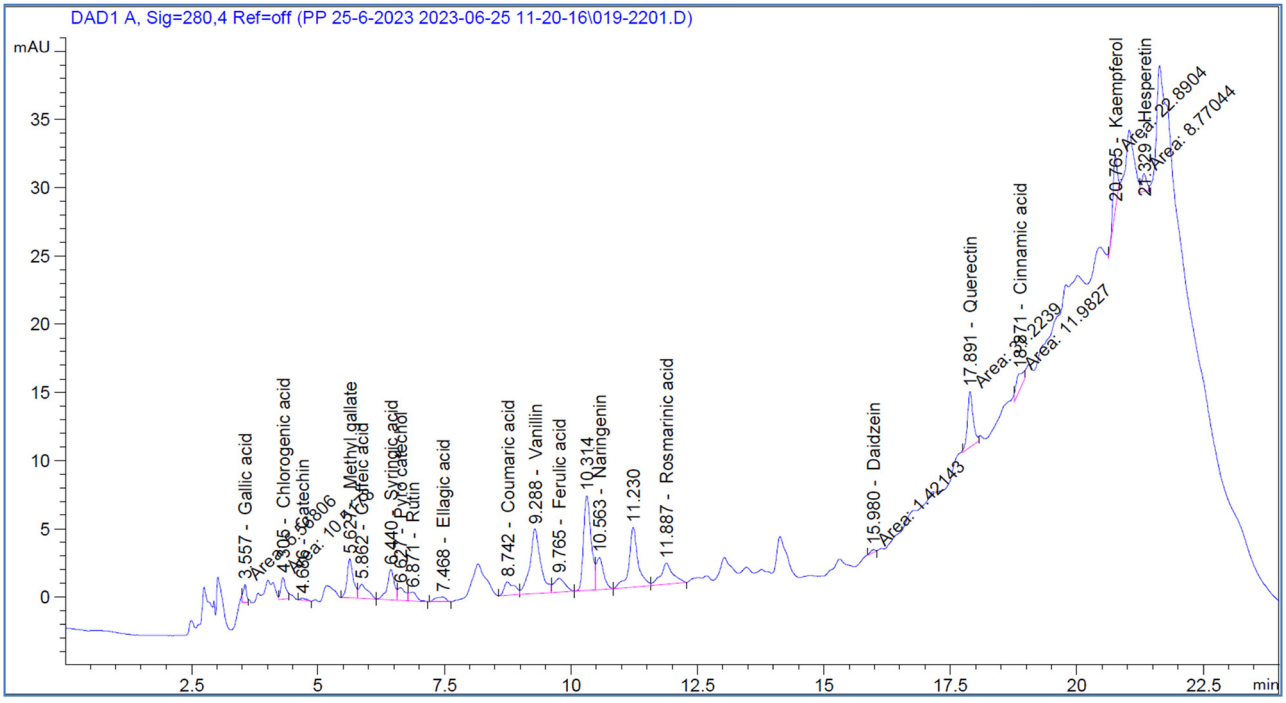

Figure 3: HPLC-phytochemical screening in *P. dactylifera* leaflets chloroform extract.

(μg g^{-1}) as shown in Table 2. The phenolic compounds identified, along with their concentrations (μg g^{-1}), included mainly coumaric acid (1663.91), gallic acid (967.12), chlorogenic acid (546.94), vanillin (490.93), methyl gallate (297.49), pyrocatechol (247.82), syringic acid (247.80), caffeic acid (222.99), catechin (212.41), ferulic acid (175.53), ellagic acid (90.78), and rosmarinic acid (36.26) in the PDML extract.

Rosmarinic acid (291.08 μg g^{-1}), vanillin (268.13 μg g^{-1}), syringic acid (156.93 μg g^{-1}), chlorogenic acid (144.67 μg g^{-1}), pyrocatechol (139.34 μg g^{-1}), methyl gallate (129.60 μg g^{-1}), caffeic acid (104.97 μg g^{-1}), ferulic acid (93.45 μg g^{-1}), coumaric acid (51.55 μg g^{-1}), gallic acid (48.26 μg g^{-1}), ellagic acid (45.34 μg g^{-1}), and catechin (30.14 μg g^{-1}) were identified as the main phenolic compounds in the PDCL extract. The PDML extract contained various flavonoids, with the following descending concentrations: naringenin (384.82 μg g^{-1}), kaempferol (382.78 μg g^{-1}), rutin (183.25 μg g^{-1}), hesperetin (154.59 μg g^{-1}), quercetin (128.64 μg g^{-1}), cinnamic acid (88.47 μg g^{-1}), and daidzein (13.94 μg g^{-1}). In contrast, the PDCL extract exhibited quercetin (386.70 μg g^{-1}), naringenin (229.93 μg g^{-1}), kaempferol (151.32 μg g^{-1}), rutin (105.59 μg g^{-1}), hesperetin (45.12 μg g^{-1}), cinnamic acid (22.75 μg g^{-1}), and daidzein (8.35 μg g^{-1}) as the most abundant flavonoids.

3.4 GC–MS profile of *P. dactylifera* leaflets chloroform extract

GC–MS screening analyzed the PDCL extract and identified 17 bioactive compounds (Figure 4). These compounds showed similarities with the Wiley Registry 8E, Replib, and Mainlib libraries in terms of their retention duration and relative abundance area (%) (Table 3). The PDCL extract contains several beneficial compounds, with the largest abundance being *cis*-13-octadecenoic acid, methyl (26.16%), 9,12-octadecadienoic acid (*Z,Z*)-, methyl ester (22.21%), ç-tocopherol (9.59%), á-sitosterol (9.31%), and hexadecanoic acid (7.64%). These compounds make up the majority of the extract.

3.5 GC–MS profile of *P. dactylifera* leaflets methanolic extract

The GC–MS screening of PDML extract yielded 31 bioactive chemicals (Figure 5), which resembled the Wiley registry 8E, Replib, and Mainlib libraries in terms of retention duration and relative abundance area (%) (Table 4). The PDML

Table 2: HPLC-phytochemical screening in methanolic and chloroform extracts of *P. dactylifera* leaflets

Detected compounds	*P. dactylifera* leaflets extracts concentration (μg g^{-1})	
	Methanolic	**Chloroform**
Phenolic compounds		
Gallic acid	967.12	48.26
Chlorogenic acid	546.94	144.67
Catechin	212.41	30.14
Methyl gallate	297.49	129.60
Caffeic acid	222.99	104.97
Syringic acid	247.80	156.93
Pyrocatechol	247.82	139.34
Ellagic acid	90.78	45.34
Coumaric acid	1663.91	51.55
Vanillin	490.93	268.13
Ferulic acid	175.53	93.45
Rosmarinic acid	36.26	291.08
Flavonoid compounds		
Rutin	183.25	105.59
Naringenin	384.82	229.93
Daidzein	13.94	8.35
Quercetin	128.64	386.70
Cinnamic acid	88.47	22.75
Kaempferol	382.78	151.32
Hesperetin	154.59	45.12

extract contained major abundant compounds that were adopted by their highest abundant area percentages, such as methyl 9-*cis*,11-*trans*-octadecadienoate (9.96%), *trans*-13-octadecenoic acid, methylester (13.28%), 1-heptatriacotanol (9.82%), ethyl iso-allocholate (9.82%), ç-sitostenone (15.28%), and acetamide, *n*-[2-(acetyloxy)-2-[3,4-bis(acetyloxy)phenyl] e-*n*-methyl (13.71%).

4 Discussion

As far as we know, recent phytochemistry reviews have increasingly emphasized the optimization of utilizing bioactive compounds derived from plants [3]. In line with this new approach, the date palm, *P. dactylifera* L. leaflet extract, is worthwhile to investigate for its phytochemical screening for further antifungal considerations [5,7]. However, previous phytochemistry studies have not comprehensively assessed the bioactive screening of date palm extracts against the growth activity of various oomycetes or necrotrophic fungi [9]. In the current study, chloroform (PDCL) and methanol (PDML) extracts of date palm leaflets were characterized and evaluated for antifungal activity against three molecularly characterized fungi. The three fungi were *F. oxysporum*, *R. solani*, and *B. cinerea* and were deposited in GenBank under accession numbers of OR116511, OR116530,

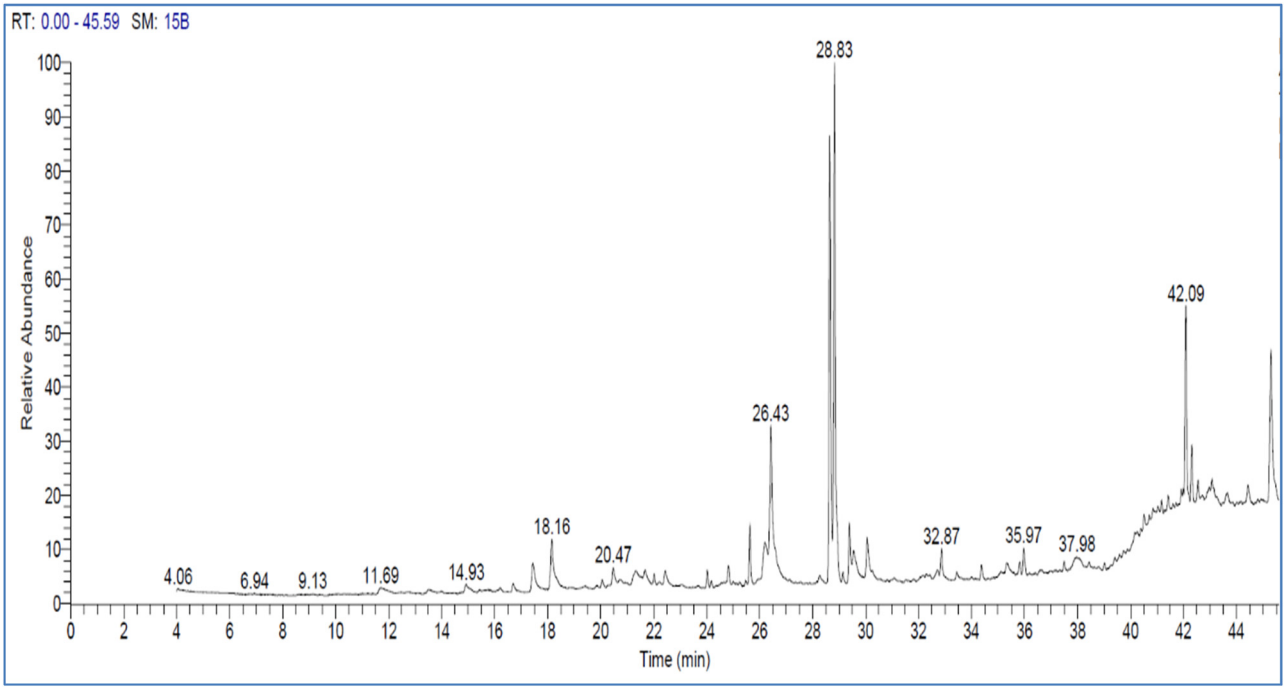

Figure 4: GC–MS phytochemical screenings of *P. dactylifera* leaflets chloroform extract.

Table 3: GC–MS phytochemical screening of *P. dactylifera* leaflets chloroform extract

RT (min)	Relative abundance%	Compounds	Class
17.44	1.92	8*H*-pyrido[1,2-*a*]pyrazin-8-one,1,2,3,4-tetrahydro-9-hydro xy-1-methyl-	Bicyclic hydroxypyridone
18.16	2.84	Dodecanoic acid	Fatty acid
24.02	0.88	2-Pentadecanone, 6,10,14-trimethyl-	Ketone
24.82	0.97	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Flavonoid
25.63	2.87	Hexadecanoic acid, methyl ester	Fatty acid ester
26.17	2.26	*trans*-Sinapyl alcohol	Monolignols
26.43	7.64	Hexadecanoic acid	Fatty acid
28.65	22.12	9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester	Fatty acid ester
28.83	26.16	*cis*-13-Octadecenoic acid, methyl	Fatty acid ester
29.39	2.77	Octadecanoic acid, methyl ester	Fatty acid ester
30.06	2.37	Octadecanoic acid	Fatty acid
32.87	1.48	Isochiapin B	Sesquiterpene lactone
35.97	1.58	Ethanol,2-(9-octadecenyloxy)-, (*z*)-	Alcohol
42.09	9.59	ç-Tocopherol	Vitamin E
42.31	2.75	9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-, 3-acetate	Steroid
42.55	2.48	Stigmast-5-en-3-ol, (3á,24s)-	Phytosterols
45.30	9.31	á-Sitosterol	Phytosterols

and OR116493, respectively. At a concentration of 150 µg mL^{-1}, PDCL and PDML demonstrated pronounced inhibitory effects on the growth of all fungal isolates, particularly *F. oxysporum* and *B. cinerea*. Therefore, the HPLC polyphenols found in both extracts are likely responsible for inhibiting the growth activity of the selected fungal isolates. Previous studies have demonstrated that rosmarinic acid is a significant phytochemical found in extracts of *Asparagus officinalis* and *Salvia rosmarinus*. These extracts have shown strong inhibitory effects against *F. oxysporum* [29], *B. cinerea*, and *R. solani* [15].

Chlorogenic acid produced by the grafted root of watermelon may inhibit the mycelial growth of *F. oxysporum* [30], *R. solani* [31], and *B. cinerea* in the rhizosphere of *Cucumis sativus* seedlings [32]. Free gallic acid could inhibit the growth of *F. oxysporum* [33]. Gallic acid, among the abundant phenols in the leaf extracts of wild

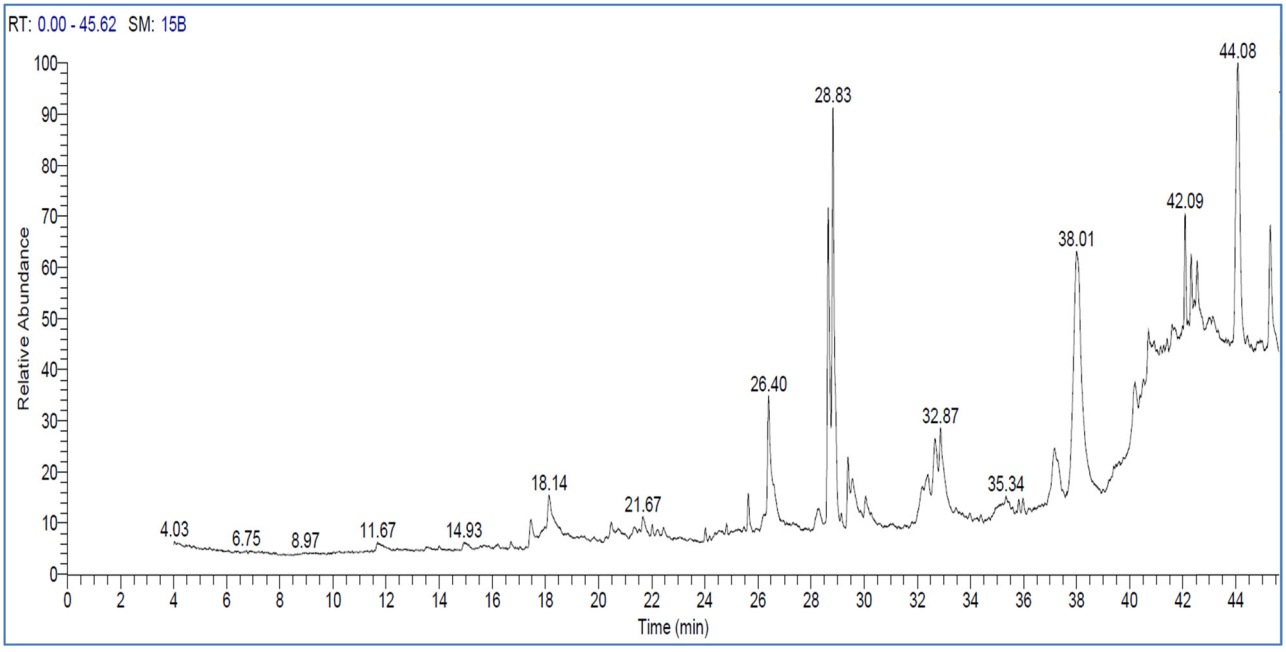

Figure 5: GC–MS phytochemical screening of *P. dactylifera* leaflets methanolic extract.

Table 4: GC–MS phytochemical screening of *P. dactylifera* leaflets methanolic extract

RT (min)	Relative abundance (%)	Compounds	Class
18.13	1.44	Dodecanoic acid	Fatty acid
20.48	0.6	(*E*)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	Methoxyphenols
21.66	0.76	Neocurdione	Sesquiterpene
22.02	0.37	9-Oximino-2,7-diethoxyfluorene	Organic compound
22.44	0.39	Dasycarpidan-1-methanol,acetate (ester)	Alkaloid
24.02	0.41	9-Oximino-2,7-diethoxyfluorene	Organic compound
24.82	0.30	7,9-Di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Flavonoid
25.63	1.36	Pentadecanoic acid,14-methyl-, methyl ester	Fatty acid ester
25.63	1.36	Hexadecanoic acid, methyl ester	Fatty acid ester
26.40	4.13	Hexadecanoic acid	Fatty acid
28.26	4.32	Lup-20(29)-ene-3,28-diol, (3á)-	Triterpene
28.31	0.58	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	Aldehyde
28.65	9.96	Methyl 9-*cis*,11-*trans*-octadecadienoate	Fatty acid ester
28.83	13.28	*trans*-13-Octadecenoic acid, methyl ester	Fatty acid ester
29.14	0.27	01297107001 Tetraneurin-*a*-diol	Terpene lactones
29.39	2.45	Octadecanoic acid, methyl ester	Fatty acid ester
29.55	1.60	9-Octadecenoic acid (*z*)-	Fatty acid
30.06	1.06	Digitoxin	Steroid glycoside
32.66	2.33	Lupeol	Triterpene
34.39	9.82	1-Heptatriacotanol	Alcoholic compound
35.34	9.82	Ethyl iso-allocholate	Steroid derivative
35.82	0.45	4*H*-1-Benzopyran-4-one,2-(3,4-dihydroxy phenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	Flavonoid
38.00	15.82	ç-Sitostenone	Sterol
40.19	2.12	Betulin	Triterpene
40.37	1.23	03027205002 Flavone	Flavonoid
42.09	3.46	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	Steroid
42.32	2.16	Lup-20(29)-ene-3,28-diol, (3á)-	Triterpene
1.99	1.99	9,12-octadecadienoic acid(*Z*,*Z*)-	Fatty Acyls
44.08	13.71	Acetamide,*n*-[2-(acetyloxy)-2-[3,4-*bis* (acetyloxy)phenyl] ethyl]-*n*-methyl-	Organic compound
44.86	0.16	Tricyclo[20.8.0.0(7,16)]triacontane,1(22),7(16)-diepoxy-	Terpene

grapevines, *Vitis* spp., and *Coccoloba uvifera*, was deemed to attain inhibition against *B. cinerea* [34] and *R. solani* [35], respectively. Catechin is one of the potent HPLC-polyphenolic fractions in the leaf extract of *Pinus wallachiana* that exhibited inhibition on the mycelial growth of *F. oxysporum* f. sp. *cubense* [36]. Free epicatechin inhibited the growth of the apple gray mold, *B. cinerea*, by modulating the phenylpropane metabolism pathway [37]. Potent growth inhibition against *F. oxysporum* could be accomplished by catechol-type siderophores produced by treatment with *Pseudomonas syringae* BAF.1 [38]. After 3 days of incubation, free catechol could bring about a diminishing of mycelia masses and sizes in *R. solani* [39]. Meanwhile, pyrocatechol produced by *Acinetobacter calcoaceticus* HIRFA32 and *Pseudomonas fluorescens* Mst8.2 inhibited the mycelial growth of *B. cinerea* [40,41]. Isomers of *o*-coumaric, *m*-coumaric, and *p*-coumaric suppressed the growth of *F. oxysporum* [42]. Coumaric acid gave rise to more than 20% inhibitory effects against *B. cinerea* [40] and complete inhibition against *R. solani* [43].

However, certain HPLC flavonoids detected in both tested extracts were found to have the ability to impede the growth of the three fungi. Numerous studies have confirmed the antibacterial properties of rutin, a phytochemical present in the peel extracts of *Musa paradisiaca* against *R. solani* [35]. Caffeic acid has shown restricted antifungal activity against *F. oxysporum* [29] and has an inhibiting effect on *B. cinerea* [37]. Low concentrations of syringic acid made *Fusarium* more common in the rhizosphere of *C. sativus* seedlings, but it did not have much of an effect on the growth of *B. cinerea* [44]. The presence of ferulic acid in leaf extracts from wild grapevine *Vitis* spp. may cause growth inhibition against *F. oxysporum* and *B. cinerea* [34,42]. In addition, certain phytochemicals, such as vanillin [45] and *trans*-cinnamic acid [46], enhance the

permeability of the pathogen's membrane in *B. cinerea*, thereby inhibiting its mycelial proliferation. A coating of chitosan and vanillin can make tomato fruit last longer and keep it safe, even when it is exposed to *F. oxysporum*, while it is being stored at room temperature and a humidity level of 60 ± 5% [47]. Methyl gallate and free cinnamic acid have a notable inhibitory effect on the mycelial development and structure of *F. oxysporum* [48]. Because they contain ellagic acid, peel extracts of *M. paradisiaca* may be able to inhibit *R. solani* [35]. Additionally, laboratory experiments conducted on the produced compounds derived from alkali gallate esters have shown significant potential in controlling *R. solani* [49].

From the GC–MS results, it was found that the long-chain saturated fatty acids (SFAs) in the PDML extract (hexadecanoic acid (C16:0), pentadecanoic acid, 14-methyl-, methyl ester (C17:0), and octadecanoic acid, methyl ester (C19:0)) and the PDCL extract (hexadecanoic acid, methyl ester (C17:0), hexadecanoic acid (C16:0), octadecanoic acid, methyl ester (C19:0), and octadecanoic acid (C18:0)) were able to inhibit the growth of the tested fungal isolates. Our assumption was confirmed by Guimarães and Venâncio [50], who elucidated that long-chain SFAs have a high number of hydrophobic groups that promote the interaction with the cell membrane. Moreover, GC fractions of straight medium-chain SFAs in PDCL and PDML extracts, including dodecanoic acid (C12:0), might even pose at least a slight inhibition against the tested isolates. These findings were followed the elucidation that the medium-chain SFAs (C7-12:0) could attain antimicrobial action with MICs of 100–200 μg mL^{-1} and biofilm formation at 2 μg mL^{-1} with 75% inhibition [50]. On the other hand, the good availability of the long-chain *cis*-unsaturated fatty acids (*cis*-UFAs) in the extracts of PDML (9-octadecenoic acid (*Z*)- (C18:1) and 9,12-octadecadienoic acid (*Z,Z*)- (C18:2), as well as in PDCL (9,12-octadecadienoic acid (*Z,Z*)-, methyl ester (C19:2), and *cis*-13-octadecenoic acid, methyl ester (C19:1), is thought to possess inhibitory effects on the tested isolates. This speculation matches the prior demonstration that the more numerous *cis*-UFAs (lower thermodynamic stability) than *trans*-UFAs have a higher influence on the cell membrane of targeted microorganisms [50]. Other GC–MS minor fractions, such as 2-pentadecanone, 6,10,14-trimethyl-, and isochiapin B in PDCL extract, may display inhibitory effects on the tested isolates. Prior research had educated that 2-pentadecanone, 6,10,14-trimethyl- of *Datura metel* extract might cause antifungal activity against *R. solani* [51]. The pigment isochiapin B produced from *Epicoccum nigrum* inhibited the biological activity of *F. solani* [52]. Likewise, lupeol and ethyl iso-allocholate in PDML extract might pose growth inhibition to the tested isolates. Several investigations have been reported on botanical extracts that suggest rolling antifungal activity due to specific abundant GC–MS fractions, such as lupeol against *F. oxysporum* [53] and *B. cinerea* [54]. Likewise, ethyl iso-allocholate has been reported against *F. oxysporum* [55]. Further studies on the isolation and characterization of these bioactive compounds represent pivotal stages in the ongoing advancement of botanical fungicides, presenting ecologically sustainable and health-conscious substitute alternatives to traditional chemical fungicides.

5 Conclusion

In conclusion, the analysis of *P. dactylifera* L. leaflet extracts with chloroform (PDCL) and methanol (PDML) reveals significant differences in the composition of phenolic compounds and flavonoids between the PDML and PDCL extracts. The potential mode of action of the compounds identified in the PDML and PDCL extracts involves their inhibitory effects on the growth of *F. oxysporum* and *B. cinerea*. Specifically, prominent phenolics like coumaric and rosmarinic acids and flavonoids such as naringenin, kaempferol, and quercetin, along with fatty acid derivatives like methyl 9-*cis*-11-*trans*-octadecadienoate and *cis*-13-octadecenoic acid, are implicated in this inhibition. These compounds likely interfere with vital biological processes or metabolic pathways in the fungi, leading to growth inhibition. The distinct chemical profiles of the PDML and PDCL extracts contribute to their diverse biological activities against the tested fungi, suggesting potential applications in controlling fungal diseases in date palm fields.

Acknowledgments: The authors express their sincere thanks to the City of Scientific Research and Technological Applications (SRTA-City) and the Faculty of Agriculture (Saba Basha), Alexandria University, Egypt, for providing the necessary research facilities. The authors would like to extend their appreciation to the Researchers Supporting Project number (RSP2024R505), King Saud University, Riyadh, Saudi Arabia.

Funding information: The research was financially supported by Researchers Supporting Project number (RSP2024R505), King Saud University, Riyadh, Saudi Arabia.

Author contributions: Study conception and data collection: K.A.H., S.B., H.H.E., and A.A.; analysis, writing results, and drafting the manuscript, K.A.H., S.B., A.A., P.K., H.H.E., and A.Al. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animals use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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