

Phytochemical composition and antifungal effectiveness of *Phoenix dactylifera* L. rachis extracts

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The present study appraised the inhibitory role of ethanol (PDEE) and ethyl acetate (PDEAE) extracts of *Phoenix dactylifera* L. against three molecularly identified fungi: *Fusarium oxysporum*, *Botrytis cinerea*, and *Rhizoctonia solani*. HPLC analysis revealed that gallic acid was the major phenolic compound in both extracts: (PDEE: 1721.90 µg/g) and (PDEAE: 101.53 µg/g). The major flavonoids in PDEE are rutin, kaempferol, and quercetin, whereas PDEAE contains kaempferol, naringenin, and quercetin. The GC-MS showed 11-octadecenoic acid methyl ester (26.25%) is the highest compound in PDEE, while diisooctyl phthalate (18.82%) is the most important compound in PDEAE. At 50 µg/mL, the inhibition percentage of PDEAE initiated the highest growth inhibition of *F. oxysporum* (49.63%) and *R. solani* (71.43%). Meanwhile, PDEE at 200 µg/mL initiated an inhibition value of 77.78% for *B. cinerea*. As a result, PDEAE is considered more effective than PDEE in controlling the growth of selected isolates.

Keywords: *Phoenix dactylifera* L., antifungal activity, HPLC, GC-MS, gallic acid, 11-octadecenoic acid methyl ester.

INTRODUCTION

The rise in multi-drug-resistant pathogens and pernicious side effects of conventional fungicide usage have increased the demand for the recognition of botanical-origin bioactive components. In terms of ease of access, frugality, and social legacies, the World Health Organization deduced that 80% of economically limited nations have previously used conventional plant-origin therapies. Notwithstanding, plants therapeutically still face a lake of phytochemical novelty compared to traditional chemical traits^{1,2}. The renaissance in phytochemistry technicality increases the outstanding usage of isolated bioactive compounds from plant origin or their synthetic counterparts³.

The date palm tree, *Phoenix dactylifera* L., is a monocotyledon angiosperm and is grown as a tiptop-diameter crop in Arabian Peninsula, North Africa, Middle East and USA^{4,5}. Rachis, well-known as the fruit stalk in female trees, is the flat or tapering peduncle part on female trees that bears flowers and fruits. The strand of inflorescence comprises numerous upright rachillae, which are spirally lined on the rachis⁶. The phytochemical profiles of eight rachis extracts of *P. dactylifera* were investigated, demonstrating the presence of bioactive components such as flavonoids, tannins, alkaloids, and coumarins that separate them from other substances. Both the dichloromethane rachis extract of “Bent-Cherk” and the ethyl acetate rachis extract of “Rotbi” showed strong antifungal activity. The in vivo test showed that the major polyphenols in these extracts exhibited some resemblance in resistance, sensibility, and natural defense against *Fusarium oxysporum* f. sp. *albedinis*⁷. Previous

studies have only investigated the bioactive compounds of date palm extracts that affect fungal growth activity, particularly on oomycetes or necrotrophic dominations^{7,8}.

Copper (II) formate, among copper complex fungicides, has been reported for its synergistic effect between copper and formaldehyde^{9,10}. Copper (II) formate complex is a copper salt or ester of formic acid. It has been used for a variety of purposes, including antimicrobial coatings and other agro-applications. A while ago, copper formate attained cross-linking with the cellulose of cotton fabrics to realize a long-lasting rot-proof against cellulolytic organisms^{9,11}. It was reported that copper formate at a concentration of 200 µg/mL effectively slowed down the growth rate of *Rhizoctonia solani*¹². As a result, a complex composed of picro-cupric ammonium formate reduced *B. cinerea*'s capacity to infect *Rosa hybrida* flowers after they had been plucked at 1000 mg/mL¹³. Amer et al.¹² reported that *R. solani*, *F. oxysporum*, and *B. cinerea* are examples of high-risk pathogens that affect strawberry crop yield around the world. The soilborne fungus *F. oxysporum* is a saprophytic fungus renowned for promoting wilt or root rot¹⁴. Moreover, one of the most important soilborne fungi amongst the genus *Rhizoctonia* is *R. solani*, which gave rise to a brown rot of stems. Its culture possesses remarkable morphological variability, a wide host range, and high pathogenicity¹⁵. The gray mold, *B. cinerea*, is known as polyphagous and one of the most serious phytopathogenic fungi. Its variable genotype and phenotype afford perfect adaptation to the environment^{16,17}. In this regard, our investigation focuses on the inhibitory effects of *P. dactylifera* rachis extracts with ethanol (PDEE) and ethyl acetate (PDEAE)

compared to copper formate complex on the mycelial growth of the fungal strains *F. oxysporum*, *B. cinerea*, and *R. solani*. The phytochemical profiles of these extracts were analyzed using HPLC and GC-MS to determine the relationships between their bioactive components and their antifungal activity.

MATERIALS AND METHODS

Source of fungal strains

In this study, we use three fungal isolates that were previously isolated at 2023 from strawberry plants namely *Botrytis cinerea* isolate BC-101 (accession number, OR116486), *Fusarium oxysporum* isolate FO-93 (accession number, OR116505) and *Rhizoctonia solani* isolate RHS-294 (accession number, OR116525) which were identified using primers ITS1 and ITS4¹². All the fungal strains were maintained on potato dextrose agar (PDA) slants at 4 °C.

Extraction procedure of *Phoenix dactylifera* L.

The date palm (10 years old), *Phoenix dactylifera* L., were sampled from Borg El Arab City, Alexandria, Egypt, at coordinates 30°49'02.3"N 29°30'50.0"E. *Phoenix dactylifera* L. rachis was left for dryness at 25 °C for two weeks. An ultrafine powder of the dried rachis was emitted by a grinder machine (Lab Universal Grinder FW100, Carlsson Technologies Sdn Bhd, Malaysia). One hundred grams of the ultrafine powder of rachis were extracted by 500 mL of high purity-solvents (96%) of each ethanol and ethyl acetate for a week under room temperature conditions. Then, the extract solution was filtered using Whatman® qualitative filter paper, Grade 1 (cellulose filter circles, 0.25 psi wet burst, 150 sec/100 mL speed (Herzberg), diameter 90 mm, thickness 180 µm, pore size 11 µm, Germany). The filtrated solvent was discarded under a vacuum process with agitating using a rotary evaporator at 40 °C to obtain the crude extract residue. The crude extracts were packed and tightly sealed into glass vials under fridge conditions at 4 °C¹⁸. At the time of experiments, the stored vials were called up anew to prepare a series of concentrations of extract-water solutions. To maintain the homogeneity of these solutions, dimethyl sulfoxide (DMSO) was added for emulsification.

Inhibition potency of *Phoenix dactylifera* L.

The technique of radial growth activity upon poisoned food was performed by Kumar et al.¹⁹ to estimate the inhibitory effect of *P. dactylifera* rachis extracts against the mycelial growth activity of the selected isolates. Plates of rachis extract's doses-PDA at 50, 100, 200, and 300 µg/mL were evaluated compared to DMSO-PDA (-ve control) and fosetyl aluminum-PDA (+ve control) at 150 µg/mL. As a positive control, copper formate (Norma CU-98; Agrotion Agricultural Investment and Industry, Egypt; a fungicide product) and copper oxychloride (Copper-Z 85% WP, Kafr El Zayat Pesticides and Chemicals, Egypt; a fungicide product) were applied at a dose of 150 µg/mL corresponding to its recommended dosage rate. One circular disc (diameter: 5 mm) of the selected fungal strain (5 days old) was laid on each PDA plate and

kept for 5 days in an incubator at 25 °C. The experiment was replicated three times. The dose-growth response of the fungi in extract-PDA compared to PDAs treated with copper and DMSO, was determined by measuring their mycelial diameters. According to the formula of Dissanayake²⁰, the inhibition percentages were expressed by the differences in growth diameters between the treatments and the negative control as follows:

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

Where, P and D, are the mycelial growth length (mm) in the negative control and the treatments, respectively.

High-performance liquid chromatography (HPLC) analysis of rachis extracts

The phytochemical profiles of PDEE and PDEAE extracts were compared to an index of 19 HPLC-standard compounds. Phytochemical screening of the crude extracts was performed by an HPLC-Agilent 1260 series. An Eclipse C18 column (4.6 x 250 mm i.d., 5 µm) performed the separation process. The mobile phase flowed at a rate of 0.9 mL/min in a linear gradient sync between two phases of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The sync program was regulated at intervals of 0 min (82:18) and up to 5 min (80:20), 8 min (60:40), 12 min (60:40), 15 min (82:18), 16 min (82:18), and 20 min (82:18) for the A: B phases, respectively. A multi-wavelength detector was fine-tuned at 280 nm. The injection volume was 5 µL of the crude extract sample. The column temperature was adjusted to 40 °C.

Gas chromatography-mass spectrometry (GC-MS) analysis of rachis extracts

The GC-MS Agilent 7000D (Agilent Technologies, Santa Clara, CA, USA) was employed to analyze crude extracts of rachis. This GC-MS Agilent 7000D was equipped with a column packed with dimethylpolysiloxane: diphenyl at 95:5, while the capillary column type was HP-5MS. The carrier gas, helium, with a purity of 99.99%, flowed at a rate of 1 mL/min. 70 eV of the ionization energy was scanned for 0.2 seconds. The detection limit of fragments ranged from 40 to 600 m/z. One µL of the crude extract sample was injected in a split ratio of 10:1 at 250 °C. The temperature ramping of the column's oven began at 50 °C with a heating rate of 3 °C/min, increased from 50 to 280 °C at a rate of 10 °C/min, and concluded at 300 °C with a heating rate of 10 °C/min. The phytochemical profile of the crude extracts was emulated with a list of compounds in the libraries of Wiley Registry 8E, Replib, and Mainlib²¹.

Statistical analysis

All the given data from laboratory tests were subjected to variance analysis (one-way ANOVA). Using the software of the Statistical Analysis System (SAS), means were significantly differentiated at the LSD 0.05 test.

RESULTS

Growth response of fungal isolates to rachis extracts of *Phoenix dactylifera*

The obtained results of the dose-growth response in the PDEE extract-PDA (Table 1) and PDEAE extract-PDA

Table 1. Dose-growth response of fungal isolates to the ethanolic extract of *Phoenix dactylifera* L. rachis compared to copper formate after 5 days of incubation

Concentration (µg/mL)	Growth [diameter (mm) ± SD ¹ ; inhibition (%)]					
	<i>Fusarium oxysporum</i>		<i>Botrytis cinerea</i>		<i>Rhizoctonia solani</i>	
50	32.67 ± 0.40 b	26.32	23.00 ± 0.17 b	48.89	26.67 ± 0.40 b	38.46
100	36.33 ± 0.64 b	18.05	11.00 ± 0.00 dc	75.56	21.33 ± 0.06 c	50.77
200	26.00 ± 0.17 c	41.35	10.00 ± 0.00 d	77.78	20.00 ± 0.00 c	53.85
300	23.33 ± 0.12 c	47.37	10.33 ± 0.06 d	77.04	20.00 ± 0.00 c	53.85
-ve control ²	44.33 ± 0.06 a	0.00	45.00 ± 0.10 a	0.00	43.33 ± 0.06 a	0.00
+ve control ³	20.67 ± 0.23 c	53.38	12.00 ± 0.00 c	73.33	27.33 ± 0.29 b	36.92

¹Standard deviation.²Dimethylsulfoxide.³Copper formate (150 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}.**Table 2.** Dose-growth response of fungal isolates to the ethyl acetate extract of *Phoenix dactylifera* L. rachis compared to copper oxychloride after 5 days of incubation

Concentrations (µg/mL)	Growth [diameter (mm) ± SD ¹ ; inhibition (%)]					
	<i>Fusarium oxysporum</i>		<i>Botrytis cinerea</i>		<i>Rhizoctonia solani</i>	
50	22.67 ^d ± 0.12	49.63	23.67 ^e ± 0.12	48.92	13.33 ^d ± 0.12	71.43
100	28.00 ^e ± 0.17	37.78	29.67 ^b ± 0.06	35.97	21.00 ^e ± 0.10	55.00
200	32.67 ^b ± 0.29	27.41	28.00 ^b ± 0.17	39.57	26.00 ^b ± 0.35	44.29
300	22.67 ^d ± 0.23	49.63	28.33 ^b ± 0.23	38.85	21.67 ^e ± 0.06	53.57
-ve control ²	45.00 ^a ± 0.00	0.00	46.33 ^a ± 0.12	0.00	46.67 ^a ± 0.15	0.00
+ve control ³	19.33 ^a ± 0.06	57.04	9.00 ^d ± 0.00	80.58	9.00 ^e ± 0.00	80.71

¹Standard deviation.²Dimethylsulfoxide.³Copper oxychloride (150 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}.

(Table 2) at concentrations from 50 up to 300 µg/mL compared to the positive- and negative-PDAs, were determined by measuring their mycelial diameters. Generally, all the assigned concentrations of PDEE extract and copper formate attained significant reductions in the radial growth levels of the selected isolates compared to those in the negative control (Table 1). The lowest growth diameters for *F. oxysporum* of 26.00 and 23.33 mm in PDEE extract at 200 and 300 µg/mL, respectively, were on par with the counterpart diameter (20.67 mm) in copper formate. The PDEE extract at 200 and 300 µg/mL exhibited the lowest growth diameters for *B. cinerea* at 10.00 and 10.33 mm, respectively, surpassing their counterpart in copper formate (12 mm). Potent reductions in the growth activity against *R. solani* realized by the concentrations of PDEE extract from 100 up to 300 µg/mL were significantly lower than their counterpart in copper formate (27.33 mm).

As foregoing data, all the assigned concentrations of PDEAE extract and copper oxychloride realized a significant reduction in the radial growth diameters of the selected isolates compared to the DMSO-PDA treatment (Table 2). The highest reduction effect of copper formate on the growth diameter (19.33 mm) surpassed the equally potent reductions of PDEAE extract at both 50 and 300 µg/mL with a low growth level (22.67 mm) against *F. oxysporum*. The highest reduction effect of copper oxychloride on the growth diameter of both *B. cinerea* and *R. solani* (9.00 mm) transcended PDEAE extract at 50 µg/mL of *B. cinerea* (23.67 mm) and *R. solani* (13.33 mm) (Table 2).

Regarding the previous given results, we could find out the inhibition percentage of the rachis extract accomplishing the highest growth inhibition percentages against the tested isolates (Table 1 and 2). The highest growth inhibitions in *F. oxysporum* (47.37 and 49.63 %) were initiated by PDEE extract at 300 µg/mL and PDEAE

extract at 50 µg/mL, respectively. Meanwhile, the highest growth inhibitions in *B. cinerea* (77.78 and 48.92%) were initiated by PDEE extract at 200 µg mL⁻¹ and PDEAE extract at 50 µg mL⁻¹, respectively. The highest inhibitions (53.85 and 71.43%) in *R. solani* were initiated by PDEE and PDEAE extracts at 200 and 50 µg/mL, respectively.

HPLC analysis of *Phoenix dactylifera* L. rachis extracts

Each of the PDEE and PDEAE extracts comprised 12 phenols and 7 flavonoids (Figures 1 and 2). The HPLC chromatogram of standard polyphenolic compounds is shown in Figure S1. The HPLC-phytochemicals of the tested extracts were refuted by their peak areas (%) and concentrations (µg/g) (Table 3). The highest abundant phenols in PDEE extract had major concentrations of gallic acid (1721.90 µg/g), chlorogenic acid (739.44 µg/g), and ferulic acid (565.25 µg/g). Whereas PDEAE extract, included gallic acid (101.53 µg/g), pyrocatechol (72.48 µg/g), syringic acid (64.61 µg/g), and caffeic acid (59.48 µg/g). In addition, the highest abundant flavonoids in PDEE extract were rutin (548.45 µg/g), kaempferol (252.19 µg/g), and quercetin (240.50 µg/g). Likewise, kaempferol (85.85 µg/g), naringenin (53.93 µg/g), and quercetin (58.74 µg/g) were characterized by their majority among the flavonoid group in PDEAE extract.

GC-MS profile of *Phoenix dactylifera* rachis ethanolic extract

The GC-MS profiles of PDEE extract had 15 phytochemical moieties (Figure 3) that resembled Wiley registry 8E, Replib, and Mainlib libraries and demonstrated by retention time, and relative abundance area (%) (Table 4). Phytochemicals with the highest abundant area in PDEE extract were 11-octadecenoic acid, methyl ester (26.25%), lup-20(29)-en-3-ol, acetate, (3 \hat{a})- (22.30%), and 9,12-octadecadienoic acid (Z,Z)-, methyl ester (18.56%).

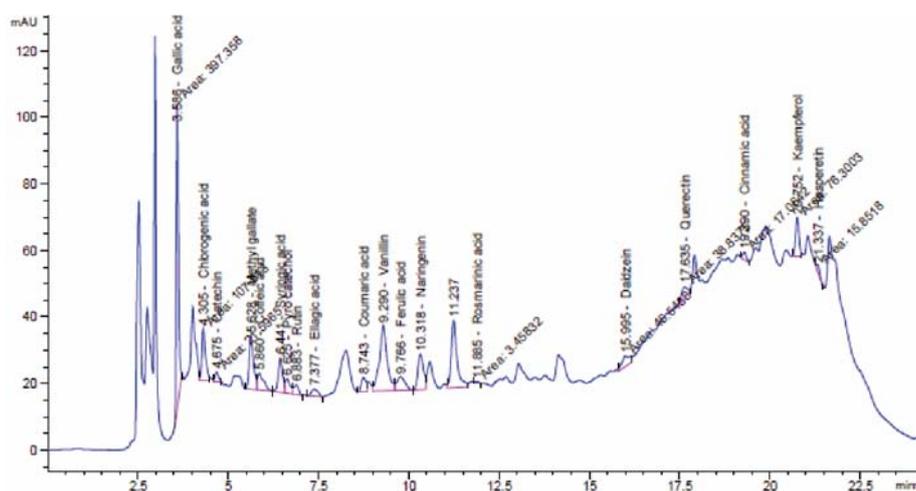


Figure 1. HPLC-phytochemical screening in *Phoenix dactylifera* rachis ethanolic extract

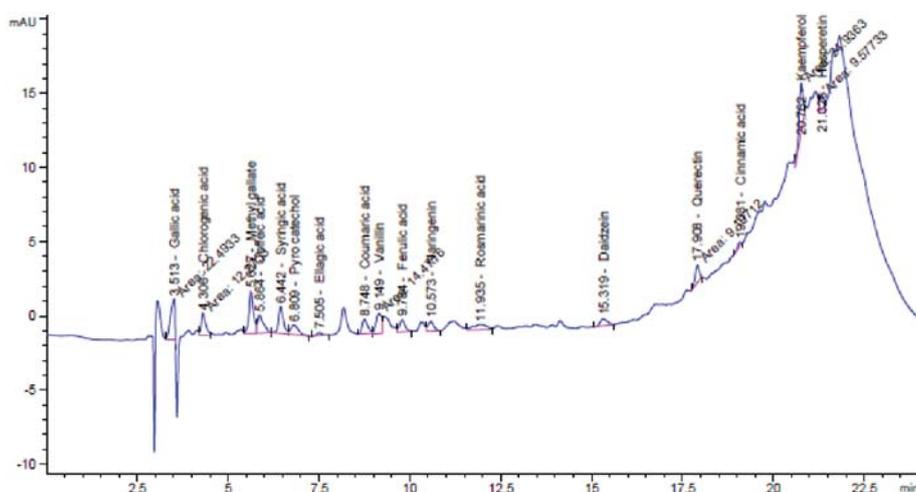


Figure 2. HPLC-phytochemical profiles in *Phoenix dactylifera* rachis ethyl acetate extract

Table 3. HPLC-phytochemical profiles in ethanolic and ethyl acetate rachis extracts of *Phoenix dactylifera* L.

Detected compound	Phytochemical profiles of <i>Phoenix dactylifera</i> rachis			
	Ethanolic extract		Ethyl acetate extract	
	Area	Concentration ($\mu\text{g/g}$)	Area	Concentration ($\mu\text{g/g}$)
Phenolic compounds				
Gallic acid	397.36	1721.90	22.49	101.53
Chlorogenic acid	107.51	739.44	12.27	87.93
Catechin	21.60	251.54	0.00	0.00
Methyl gallate	132.84	348.51	24.81	67.79
Caffeic acid	59.40	249.84	13.57	59.48
Syringic acid	86.88	317.52	16.97	64.61
Pyro catechol	42.51	307.34	9.62	72.48
Ellagic acid	27.02	202.55	0.00	0.00
Coumaric acid	26.67	119.33	2.15	10.00
Vanillin	39.32	73.75	10.54	20.59
Ferulic acid	292.59	565.25	14.47	29.12
Rosmarinic acid	65.18	200.82	8.58	27.53
Flavonoids				
Rutin	113.22	548.45	7.23	36.50
Naringenin	3.46	19.32	9.27	53.93
Daidzein	46.54	136.68	6.56	20.06
Quercetin	38.84	240.50	9.11	58.74
Cinnamic acid	17.06	16.20	1.93	1.91
Kaempferol	76.30	252.19	24.94	85.85
Hesperetin	15.85	40.77	9.58	25.66

GC-MS profile of *Phoenix dactylifera* rachis ethyl acetate extract

The GC-MS screening of PDEAE extract comprised 21 phytochemical fractions (Figure 4), which were in a resemblance to the same aforementioned libraries and demonstrated by retention time, and relative abundance

area (%) (Table 5). The PDEAE extract had phytochemical fractions with the highest abundant areas that, represented by diisooctyl phthalate (18.82%), ζ -tocopherol (12.35%), hexadecanoic acid (11.10%), 9-octadecenoic acid, methyl ester (E)- (8.87%), α -sitosterol (8.41%), dotriacontane (7.00%), and 9,12-Octadecadienoic acid (Z,Z)-,methyl ester (6.07%).

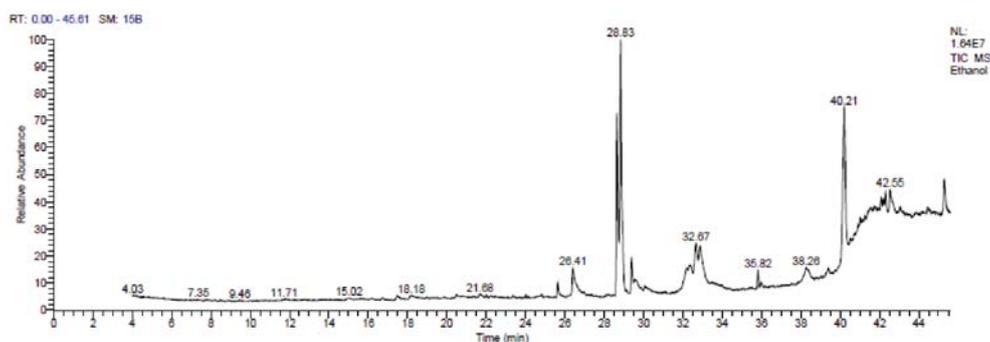


Figure 3. GC-MS phytochemical profiles of *Phoenix dactylifera* L. rachis ethanolic extract

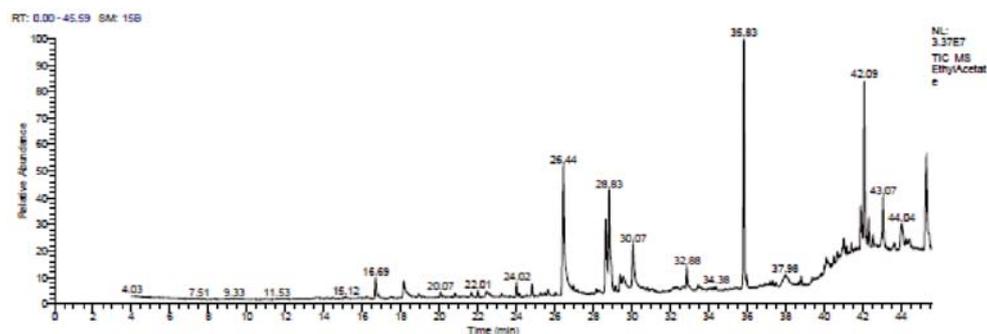


Figure 4. GC-MS phytochemical profiles of *Phoenix dactylifera* rachis ethyl acetate extract

Table 4. GC-MS phytochemical profiles of ethanolic rachis extract of *Phoenix dactylifera* L.

Retention time (min)	Relative abundance%	Phytochemical profiles
25.64	1.73	Pentadecanoic acid
26.41	3.22	Hexadecanoic acid, 2,3-dihydroxypropyl ester
28.65	18.56	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
28.83	26.25	11-Octadecenoic acid, methyl ester
29.39	3.53	Octadecanoic acid, methyl ester
32.67	7.41	Lupeol
35.82	1.77	1,2-Benzenedicarboxylic acid
40.21	22.30	Lup-20(29)-en-3-ol, acetate, (3 α)-
41.02	0.84	Silane, trimethyl[[[(3 α)-Stigmast-5-EN-3-YL]OXY]-
42.09	1.55	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3 α ,5Z,7E)-
42.19	1.29	1-Heptatriacontanol
42.31	2.53	Ethyl iso-allocholate
42.55	4.65	9,12-Octadecadienoic acid (z,z)-2,3-bis(trimethylsilyloxy)propyl ester
45.30	4.37	Stigmast-5-en-3-ol, (3 α)-

Table 5. GC-MS phytochemical profiles of *Phoenix dactylifera* L. rachis ethyl acetate extract

Retention time (min)	Relative abundance%	Phytochemical profile
16.69	1.69	3,4-Dihydro-2h-1,5-(3"-T-Butyl)benzodioxepine
18.15	1.49	Dodecanoic acid
24.02	1.12	2-Pentadecanone, 6,10,14-trimethyl-
24.82	0.95	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
26.44	11.10	Hexadecanoic acid
28.65	6.07	9,12-Octadecadienoic acid (Z,Z)-,methyl ester
28.83	8.87	9-Octadecenoic acid, methyl ester,(E)-
29.39	1.16	Octadecanoic acid, methyl Ester
30.07	3.71	Octadecanoic acid
32.88	1.87	4,8,12,16-Tetramethylheptadecan-4-olide
35.83	18.82	Diisooctyl phthalate
38.80	7.00	Dotriacontane
40.03	1.19	Hahnfett
40.51	2.85	Ethyl iso-allocholate
41.02	2.07	Isochiapin B
41.42	0.77	Betulin
41.91	2.77	17-Pentatriacontene
42.09	12.35	γ -Tocopherol
42.31	1.97	9,19-Cyclocholestene-3,7-diol,4,14-dimethyl-, 3-acetate
44.03	3.80	Astaxanthin
45.30	8.41	α -Sitosterol

DISCUSSION

As far as we know, numerous reviews on bioactive components from plant-origin have been reported on how to maximize their use in the strategy of pathogen's control for sustainable agriculture²². However, the assessment of bioactive agents in date palm extracts against the mycelial extensions of oomycetes or necrotrophic fungi still needs much more investigation⁸. In keeping with this trend, it is worthwhile to figure out the phytochemical agents in the rachis extracts of date palm, *P. dactylifera*, which may correlate with their antifungal potency^{23, 24}. As stated in our findings, all concentrations of PDEE extract and copper formate achieved significant reductions in the growth levels of the selected isolates compared to the negative control. The lowest growth activity of *F. oxysporum* in PDEE extract at 200 and 300 $\mu\text{g/mL}$ was equipollent to copper formate. The PDEE extract at $\geq 200 \mu\text{g/mL}$, and $\geq 100 \mu\text{g/mL}$ exhibited the lowest growth activity for *B. cinerea* and *R. solani*, respectively, which transcended the effect of copper formate. On the other hand, the highest reduction effect on the growth activity for *F. oxysporum* caused by copper oxychloride surpassed the equally potent reductions of PDEAE extract at both 50 and 300 $\mu\text{g/mL}$. Also, copper oxychloride had the same effect on *B. cinerea* and *R. solani*, even stronger than the 50 $\mu\text{g/mL}$ PDEAE extract.

The investigated crude extracts contain important mutual HPLC polyphenols that can inhibit the development of certain fungal isolates. Among these, free gallic acid has shown activity in inhibiting the growth of *F. oxysporum* f. sp. *niveum*²⁵. Gallic acid combined with the phyto-components of the leaf extracts of wild grapevines, *Vitis* spp., could be the reason for reducing the growth rates in *B. cinerea*²⁶, as well as in *Coccoloba uvifera* done against *R. solani*²⁷. The major exclusive HPLC-polyphenols in PDEE extract pointed out with ferulic acid, which has existed in leaf extracts of wild grapevine *Vitis* spp., were observed to increase growth diminishing of *F. oxysporum*²⁸, and *B. cinerea*²⁶. The presence of chlorogenic acid in watermelon's roots may dampen the growth of *F. oxysporum* f. sp. *niveum*²⁹, likewise, in the rhizosphere of *Cucumis sativus* seedling, it could frustrate the growth of *B. cinerea*³⁰. Additionally, chlorogenic acid had a growth restraint action on *R. solani*³¹. On the other hand, the major exclusive HPLC-polyphenols in PDEAE extract, including pyrocatechol, which has been investigated in previous studies as a by-product from *Acinetobacter calcoaceticus* HIRFA32 and *Pseudomonas fluorescens* Mst8.2, could prevent the mycelial growth of *B. cinerea*^{32, 33}. However, high doses of syringic acid could enhance *Fusarium* activity in the rhizosphere of *C. sativus* seedlings and mycelial growth diminishes on *B. cinerea* Pers^{34, 35}. An in vitro study shows that flavonoid aglycones from the roots of the *Fusarium* wilt-resistant date palm cultivar (*Phoenix dactylifera* L., cv. Takerboucht) inhibit fusaric acid synthesis and decrease the number of conidia, while those from the susceptible cultivar (Deglet Nour) stimulate mycelial growth of *Fusarium oxysporum* f. sp. *albedinis* (*F.o.a*)³⁶. Similarly, flavonoid aglycones extracted from Takerboucht cultivar leaves were highly effective in inhibiting conidiogenesis and fusaric acid production by 91.66% and 87.14%, respectively³⁷. Also, caffeic acid

could stop the activity of *F. oxysporum* f. sp. *niveum*³⁸ and *B. cinerea*³⁹. The major exclusive HPLC-flavonoid in PDEE extract indicated by rutin has been formerly studied in mixture with the phytochemicals of the peel extracts of *Musa paradisiaca* L. for its antimicrobial activity against *R. solani*²⁷.

The major exclusive HPLC-flavonoid in PDEAE extract, represented by naringenin, which has been previously evaluated in the form of an ethanolic solution, decreased the growth of *F. oxysporum*⁴⁰, and *B. cinerea* in table grapes³², beside *R. solani*²⁷. During infection of the date palm with *F.o.a*, the susceptible cultivar (Deglet Nour) accumulates para-hydroxybenzoic acid, while the resistant cultivar (Takerboucht) shows decreased levels of this acid and increased para-hydroxycinnamic acid in infested soils. The activation of trans-cinnamic acid in the roots of the resistant cultivar suggests a key role in the plant's defense against *Fusarium* wilt (Bayoud) disease⁴¹. An instance of minor HPLC-polyphenols in our crude extracts that may be the cause of the growth restraint against the selected isolates, is rosmarinic acid, which has been found among the major phytochemicals in the extracts of *Asparagus officinalis*, considered a potent reason for decreasing the growth of *B. cinerea*. Likewise, rosmarinic acid in the extracts of rosemary, *Salvia Rosmarinus*, had the same role against *R. solani*⁴² and *F. oxysporum* f. sp. *niveum*³⁸. Catechin, one of the most effective polyphenolic fractions in the leaf extract of *Pinus wallachiana*, may prevent the mycelial growth of *F. oxysporum* f. sp. *cubense*⁴². Isolated epicatechin could disrupt the phenylpropane metabolism, resulting in interception for *B. cinerea* growth³⁹. For example, catechin biosynthesized by *Trichoderma atroviride*, a bioactive agent-producing microorganism, could improve *R. solani* growth resistance in cucumber root⁴³. Catechol-type siderophores produced by *Pseudomonas syringae* BAF.1, reign a dynamic potency against *F. oxysporum* growth⁴⁴ and *R. solani*⁴⁵. Variant isomers of coumaric acid have partially lessened effects on the growth of *F. oxysporum*²⁸, *B. cinerea*³² and full inhibition against *R. solani*⁴⁶. On the other hand, some identified flavonoids in the tested extracts may act as bioactive fractions against the growth of the selected fungi, such as, vanillin⁴⁷ and trans-cinnamic acid⁴⁸, due to their potency, due to their potency to leverage the permeability to the cell membrane of *B. cinerea*. The coating application during the shelf life and storage period with chitosan and vanillin could protect the uninfected or even infected tomato fruit with *F. oxysporum*⁴⁹. Methyl gallate⁵⁰ and isolated cinnamic acid⁵¹ vigorously stopped the mycelial growth of *F. oxysporum*. In vitro trials of synthetic compounds derived from multiple alkali substitutions with gallate esters could give rise to vigor antifungal effects against *R. solani*⁵². Ellagic acid in the peel extracts of *M. paradisiaca* was considered to decline the microbial vitality of *R. solani*²⁷.

In keeping with the GC-MS data, the long-chain saturated fatty acids (SFAs) like pentadecanoic acid (C15:0) in PDEE extract, as well as hexadecanoic acid (C16:0), octadecanoic acid (C18:0) and dodecanoic acid (C12:0) in PDEAE extract are anticipated to finalize high growth inhibition against the selected isolates. As a previous interpretation performed by Guimarães and Venâncio⁵³, the longer chain of SFAs, is the more numerous hydrophobic

groups causing the interaction with the cell membrane of the pathogen. Whereas, GC-straight medium-chain SFAs (C7-12:0) represented by hexadecanoic acid, 2,3-dihydroxypropyl ester (C9:0) in PDEE extract, might cause a slight inhibition against the tested isolates. This assumption was based on previous investigations of minimum inhibitory concentration (MIC) of the medium chain SFAs that could realize 75% inhibition within the range of 100 to 200 $\mu\text{g/mL}$ ⁵³. On the other hand, the good availability of the long-chain unsaturated fatty acids (UFAs) of the PDEE extract in the cis form was 9,12-octadecadienoic acid (z,z)-2,3-bis[(trimethylsilyl)oxy (C27:2), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (C19:2), and octadecanoic acid, methyl ester (C19:2) versus a trans-UFA, 11-octadecenoic acid, methyl ester (C19:1). Likewise, PDEAE extract had a cis-UFAs, 9,12-octadecadienoic acid (Z,Z)-,methyl ester (C19:2) and octadecanoic acid, methyl Ester (C19:2) versus a trans-UFA, 9-octadecenoic acid, methyl ester,(E)- (C19:1). The cis-UFA is thought to cause inhibitory action more than trans-UFA on the tested isolates. These findings followed the aforementioned conclusion of Guimarães and Venâncio⁵³ that whenever the cis-UFAs contents exceeded the trans-UFAs, the greater the impact on the cell membrane of microbes. Minor phytochemicals, such as lupeol and ethyl iso-allochololate in PDEE extract, as well as ethyl iso-allochololate and isochiapin B in PDEAE extract might inhibit growth in our isolated fungi. As a counterpart to these phytochemicals, several studies have been carried out on botanical extracts containing abundant lupeol that concluded to display antifungal activity against *B. cinerea*⁵⁴, *F. oxysporum*⁵⁵, and *R. solani*⁵⁶. In another study is the first to analyze volatile compounds in the roots and leaflets of *Phoenix dactylifera* L. using GC-MS. Forty-one volatile substances were identified in the roots, mainly fatty acids, with methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate being the most prevalent. In the leaflets, twenty-three volatile components were found, including iso-palmitic acid and 1,3,5-benzene tricarboxylic acid, trimethyl ester. These findings highlight the potential of date palm vegetative organs as valuable sources of natural organic compounds⁵⁷. Lupeol is a potent antifungal triterpenoid and is well-known for its mitochondrial dysfunction, which leads to generating an extra rate of ROS and a minimum rate of ATP in *Saccharomyces cerevisiae*⁵⁸. Moreover, ethyl iso-allochololate has been concluded to pose antifungal activity against *F. oxysporum* f. sp. *lycopersici*⁵⁹. The by-product, isochiapin B derived from *Epicoccum nigrum*, was deemed to possess antibiological aspects against *Fusarium solani*⁶⁰.

CONCLUSIONS

The study evaluated the inhibitory potential of *Phoenix dactylifera* L. rachis extracts (PDEE and PDEAE) compared to copper formate and copper oxychloride against *Fusarium oxysporum*, *Botrytis cinerea*, and *Rhizoctonia solani*. HPLC analysis revealed gallic acid as a major phenolic compound in both extracts, while major flavonoids differed between the two. GC-MS analysis identified significant compounds in each extract. PDEAE exhibited the highest growth inhibition of *F. oxysporum*

(49.63%) and *R. solani* (71.43%) at 50 $\mu\text{g/mL}$, while PDEE showed 77.78% inhibition against *B. cinerea* at 200 $\mu\text{g/mL}$. However, copper-based controls displayed superior inhibition across all isolates, indicating PDEAE's efficacy in controlling fungal growth.

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