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Antifungal and Antibacterial Activities of *Musa paradisiaca* L. Peel Extract: HPLC Analysis of Phenolic and Flavonoid Contents

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Received: 19 March 2019; Accepted: 8 April 2019; Published: 15 April 2019

Abstract: In the present study, *Melia azedarach* wood samples that were treated with the methanolic extract of *Musa paradisiaca* L. peels were evaluated for their antibacterial and antifungal activities against *Agrobacterium tumefaciens*, *Dickeya solani*, *Erwinia amylovora*, *Pseudomonas cichorii*, *Serratia pylmuthica*, *Fusarium culmorum*, and *Rhizoctonia solani*. The strongest antibacterial activity was only found against *A. tumefaciens* (inhibition zone 90 mm), while the other bacterial strains showed resistance to wood that was treated with the extract. Potential antifungal activity against *F. culmorum* and *R. solani* was observed; the mycelial growth inhibition percentages reached 68.88% and 94.07%, respectively, in wood samples that were treated with the 3% methanolic extract of *M. paradisiaca* peel. HPLC analysis demonstrated the presence of seven phenolic compounds and three flavonoid compounds, as their peaks were matched with the standard compounds in a HPLC analysis. The major constituents of phenolic and flavonoid compounds in mg/100 g dry extract (DE) were ellagic acid (16.19), gallic acid (7.73), rutin (973.08), myricetin (11.52), and naringenin (8.47). The results demonstrated the potential effects of banana peel extract as a natural compound that can protect wood from molds while in use.

Keywords: Antifungal activity; antibacterial activity; *Musa paradisiaca* L. peels; phenolic; flavonoid; HPLC

1. Introduction

Musa paradisiaca L., growing in tropical and subtropical countries, is used globally for its nutritional value. Phytochemical screening showed that *M. paradisiaca* peel contains tannins, alkaloids, steroids, saponin, flavonoids, and carbohydrates, while cyanogenic glycoside was absent [1]. The fruit, peels, and leaves of *M. paradisiaca* are used in traditional medicine [2].

Banana peels have been reported as a good source of phenolic and flavonoid compounds [3]. The extracts of peels from different cultivars of banana were observed to have good antioxidant activity, which is correlated with the presence of phenolic and flavonoid compounds [4–7].

Among the extracts of peels from different cultivars, plantain peel flour was shown to have the lowest level of extractable polyphenols, but the highest antioxidant capacity [8]. Phenolic compounds in peel extracts of tanduk and nangka bananas, which were grown in West Java-Indonesia, were major contributors to their antioxidant activities [9]. The fatty acids that were observed in banana peel extract were responsible for its antimicrobial activity [10]. In addition, in fully ripe bananas, peel and pulp had reported to have antibiotic and antifungal properties [11].

Wood, paper, and wood products in use can be colonized by molds, causing surface discoloration when subjected to humid conditions while in use [12–17]. The phytopathogenic fungi *Fusarium culmorum*, *F. solani*, and *Rhizoctonia solani* have been reported to cause several diseases in plants, such as root rot and wilt disease complex [18,19], as well as causing postharvest problems for citrus and stone fruits [20,21].

Different plant bacterial strains, *Ralstonia solanacearum, Dickeya solani*, *Agrobacterium tumefaciens*, and *Bacillus pumilus*, have been shown to cause infectious symptoms, such as brown and soft rot in potato tubers and stems, blackleg in potatoes, and tumors in olive and other ornamental plants [22–26].

Today, new sources of biofungicides or bactericides are being rapidly developed to overcome the toxic effects of conventional pesticides. Information regarding the in vitro antibacterial and antifungal activities of *M. paradisiaca* peels extracts against plant pathogenic agents has not yet been examined. Therefore, the present study aimed to study the antimicrobial activity of wood that was treated with the extract against the growth of five bacteria strains, *Agrobacterium tumefaciens*, *Dickeya solani*, *Erwinia amylovora*, *Pseudomonas cichorii*, and *Serratia pylmuthica*; and, two fungal isolates, *Fusarium culmorum* and *Rhizoctonia solani*. HPLC was used to analyze the phenolic and flavonoid compounds that were found in methanol extract.

2. Materials and Methods

2.1. Preparation of Extract and Wood Blocks

Musa paradisiaca peels were collected from Alexandria, Egypt during 2018. The peels were air-dried under laboratory conditions and then ground into small pieces. About 50 g of the ground peels were soaked in methanol solvent (200 mL) for 3 d. and filtered using a cotton plug, followed by filter paper (Whatman No.1, Mumbai, India). Methanol was evaporated under pressure while using a rotary evaporator at 60 °C [27] to concentrate the extract. The crude methanol extract (7.15 g/100 g air-dry peels) was stored in sealed vials at 4 °C until further use. The extract was prepared at concentrations of 0, 1, 2, and 3% by dissolving in 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich). Stock solution 3% (3 g extract/100 mL of 10% DMSO) was prepared first, while the work solutions were diluted in distilled water.

The wood blocks of *Melia azedarach* were prepared at the laboratory of Wood Technology (Department of Forestry and Wood Technology, Alexandria, Egypt) with dimensions of 1 × 1 × 0.5 cm. After the preparation of the wood blocks, they were subjected to autoclaving at 121 °C for 20 min and then cooled. For the application of extract to wood samples, three wood samples were used for each concentration for each fungus or bacterium, and each wood sample received 100 µL of the concentrated extract according to our previous published studies, with minor modifications [17,28].

2.2. Antimicrobial Activity of Wood Treated with Methanol Extract

Five strains of plant bacterial pathogens Agrobacterium tumefaciens, Dickeya solani, Erwinia amylovora, Pseudomonas cichorii, and Serratia pylmuthica; and, two plant pathogenic fungi, Fusarium culmorum MH352452 and Rhizoctonia solani MH352450, were used for the bioassay. All the microorganisms were provided by the Microbiology Laboratory, Agricultural Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt.

The agar disc diffusion method [29] was used for the determination of the antibacterial activity of the methanol extract and the diameters of the inhibition zones (IZs) were measured in mm. The control samples received 100 µL of 10% DMSO. All of the tests were performed in triplicate.

The antifungal activity was measured according to our previous studies [17,30–32], where wood samples that were treated with the concentrated extract were placed over a potato dextrose agar (PDA) medium and inoculated with a 5 mm diameter disc of 7-d-old PDA culture of *F. culmorum* or *R. solani*. The incubation periods took seven days at 25 ± 1 °C. The inhibition of fungal mycelial growth was measured using the following equation:

$$\text{Mycelial growth inhibition (\%)} = [(A0 - At)/A0] \times 100, \quad (1)$$

where *A0* and *At* are the average diameters of the control and treatment fungal colonies, respectively.

2.3. HPLC Conditions for Phenolic and Flavonoid Compounds

Gallic acid, catechol, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, ellagic acid, benzoic acid, *o*-coumaric acid, salicylic acid, cinnamic acid, rutin, myricetin, quercetin, naringenin, kaempferol, and apigenin were used as standard compounds for the presence of phenolic and flavonoid compounds in the methanol extract of the *M. paradisiaca* peels. HPLC Instrument and its conditions can be found in our previous studies [17,33].

2.4. Statistical Analysis

The results of the inhibition zones against the growth of bacteria as well as the percentage of reduction in mycelial growth against the growth of fungi in treatment controls, as affected by four concentrations (0%, 1%, 2%, and 3%) of the methanolic extract of *M. paradisiaca* peels, were analyzed statistically with one-way analysis of variance (ANOVA) using SAS software SAS software (SAS Institute, Release 8.02, Cary, North Carolina State University, Raleigh, NC, USA) [34]. The means were compared against the control treatment according to the $LSD_{0.05}$ test.

3. Results

3.1. Visual Observations of Antibacterial Activity on Extract-Treated Wood

Figure 1 and Table 1 showed the antibacterial activity of wood when treated with *M. paradisiaca* peel extract. Generally, the strongest activity was observed against the growth of *Agrobacterium tumefaciens*, where no growth of the bacterium was found on wood that was treated with the extract at all of the examined concentrations, and the inhibition zone reached 90 mm. On the other hand, *Dickeya solani*, *Erwinia amylovora*, *Pseudomonas cichorii*, and *Serratia pylmuthica* showed resistance to wood that was treated with the extract, and complete growth was observed.

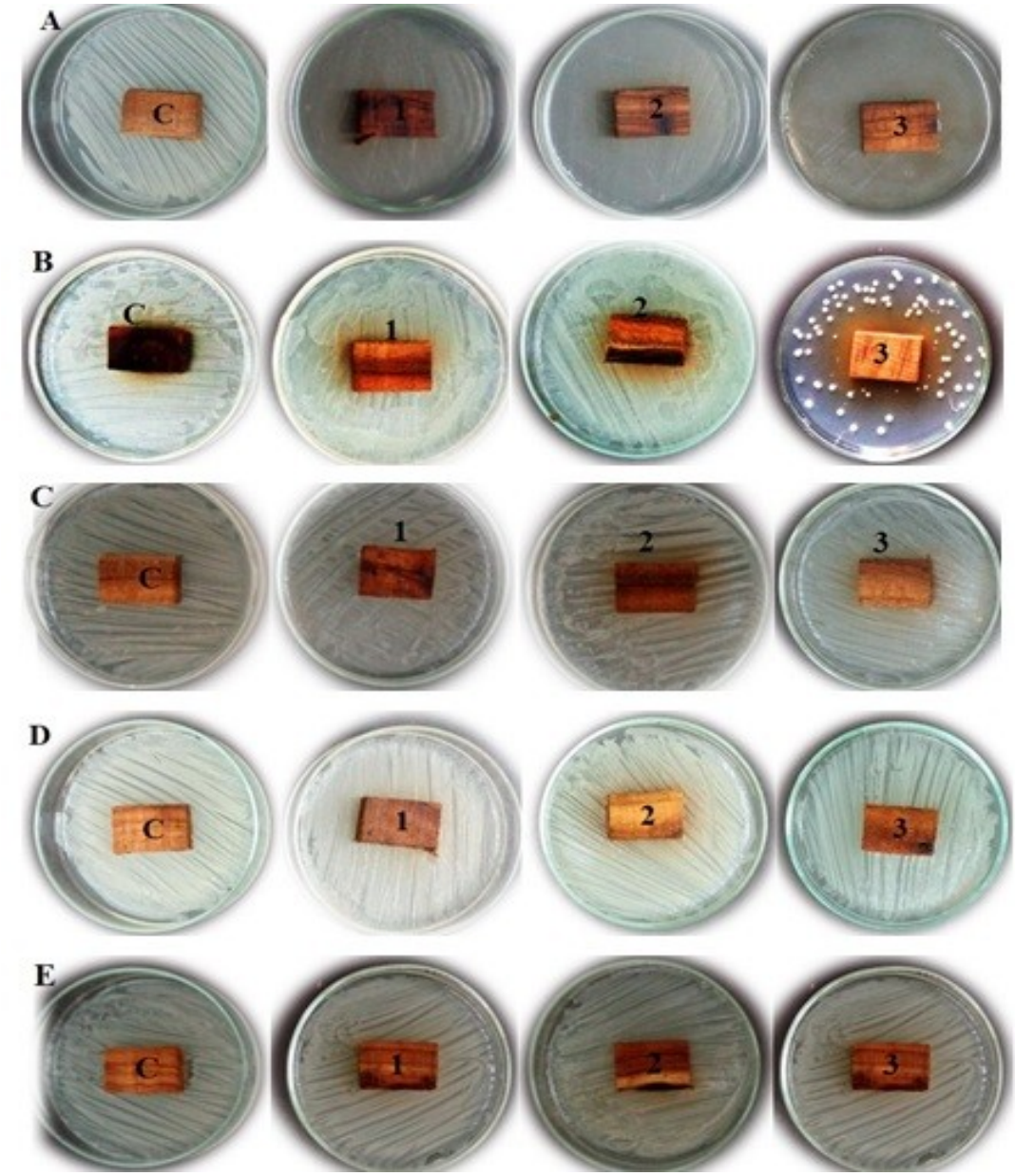

Figure 1. Antibacterial activity of wood treated with the methanoilc extract of *M. paradisiaca* peels. (**A**) *A. tumefaciens*; (**B**) *D. solani*; (**C**) *E. amylovora*; (**D**) *P. cichorii*; (**E**) *S. pylmuthica.*

Table 1. Antibacterial activity of wood that was treated with the methanolic extract of *M. paradisiaca* peel against the bacterial strains.

Conc. (%)	Inhibition Zone (mm)				
	A. tumefaciens	*D. solani*	*E. amylovora*	*P. cichorii*	*S. pylmuthica*
0	0.00 ^{b}	0.00	0.00	0.00	0.00
1	90.00 ^{a}	0.00	0.00	0.00	0.00
2	90.00 ^{a}	0.00	0.00	0.00	0.00
3	90.00 ^{a}	0.00	0.00	0.00	0.00
Significant	***	ns	ns	ns	ns

***: Highly significant; ns: not significant; Means with the same superscript letter within the same column are not significantly different according to the LSD test at a 0.05 level of probability.

3.2. Antifungal Activity of the Extract

Figure 2 shows that, with an increase in the concentration of the extract, the linear fungal growth of *Fusarium culmorum* and *Rhizoctonia solani* was decreased. The mycelial growth inhibition percentages (Table 2) of wood that was treated with the extract reached 37.03, 55.18, and 68.88% against the growth of *F. culmorum* at concentrations of 1, 2, and 3%, respectively, as compared to those of the control treatment, while it reached 50.00, 93.33, and 94.07% against the growth of *R. solani* at the same concentrations.

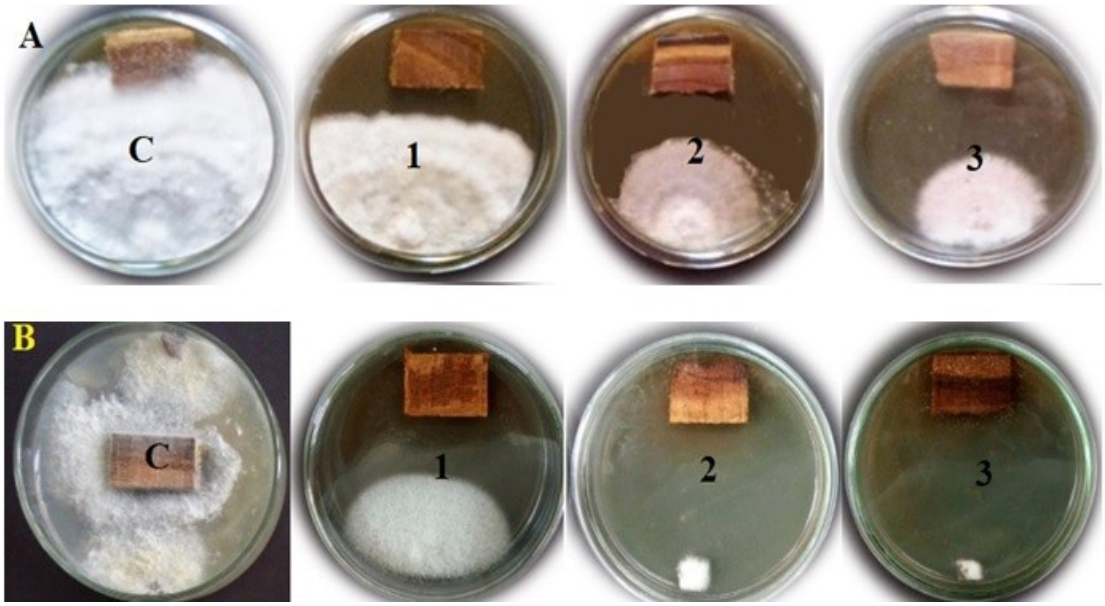

Figure 2. Antifungal activity of wood treated with the methanolic extract of *M. paradisiaca* peels. (**A**) *Fusarium culmorum*, (**B**) *Rhizoctonia solani*.

Table 2. Inhibition percentage of fungal mycilial growth of wood treated with the methanoilc extract of *M. paradisiaca* peels.

Conc. (%)	Inhibition Percentage (%)	
	F. culmorum	***R. solani***
0	0.00 ^{d}	0.00 ^{c}
1	37.03 ^{c} ± 2.79	50.00 ^{b} ± 0.00
2	55.18 ^{b} ± 1.69	93.33 ^{a} ± 1.11
3	68.88 ^{a} ± 2.22	94.07 ^{a} ± 0.64
$LSD_{0.05}$	3.722	1.207

Means with the same superscript letter within the same column are not significantly different according to the LSD test at a 0.05 level of probability.

3.3. Phenolic and Flavonoid Compounds of the Methanol Extract

Table 3 presents the phenolic (Figure 3) and flavonoid (Figure 4) compounds that were identified in the methanolic extract of *M. paradisiaca* peels. The phenolic and flavonoid compounds in the concentration of mg/100 g DE and matched with the standard as analyzed by HPLC were ellagic acid (16.19), gallic acid (7.73), ferulic acid (1.63), *o*-coumaric acid (1.12), catechol (0.82), salicylic acid (0.27), cinnamic acid (0.07), rutin (973.08), myricetin (11.52), and naringenin (8.47).

Table 3. Chemical composition analysis of phenolic and flavonoid compounds of the methanolic extract of *M. paradisiaca* peels by HPLC.

Compound	Conc. (mg/100 g DE *)
Phenolic compounds	
Gallic acid	7.73
Catechol	0.82
Ferulic acid	1.63
Ellagic acid	16.19
o-Coumaric acid	1.12
Salicylic acid	0.27
Cinnamic acid	0.07
Flavonoid compounds	
Rutin	973.08
Myricetin	11.52
Naringenin	8.47

* DE: dry extract.

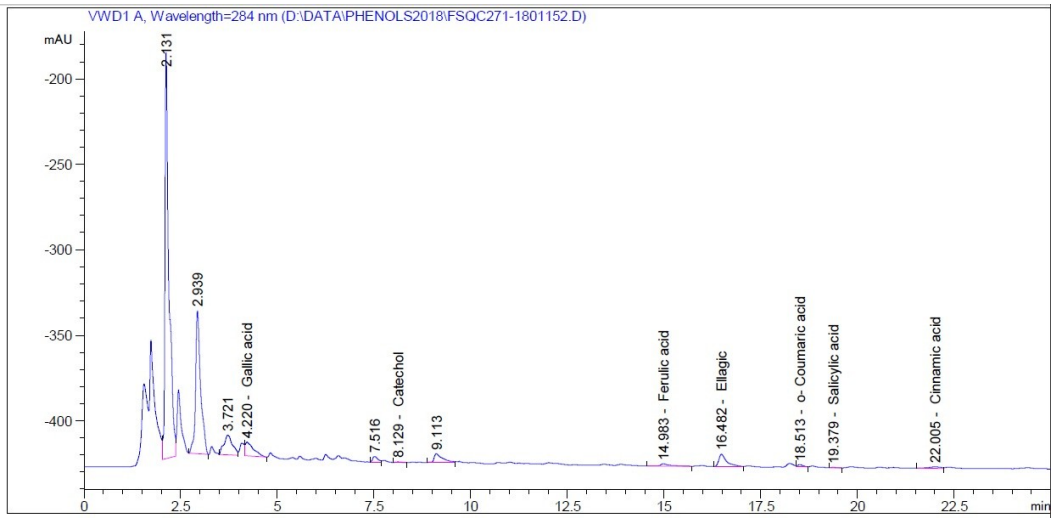

Figure 3. Chromatogram of phenolic compounds of *M. paradisiaca* methanolic peel extract analyzed by HPLC.

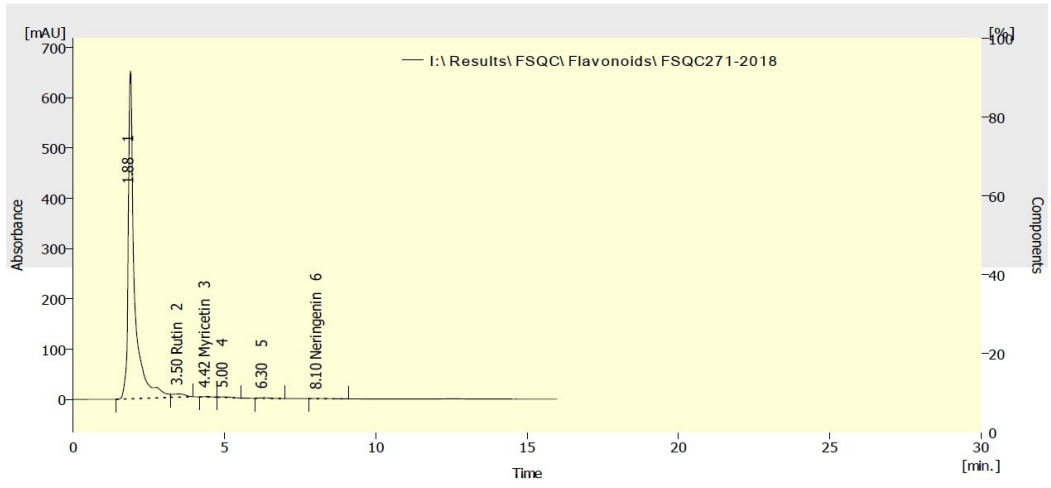

Figure 4. Chromatogram of flavonoid compounds by HPLC in methanol extract of *M. paradisiaca* peels.

4. Discussion

Phenolic and flavonoid compounds that are commonly found in plants have been reported to have potential biological effects, including antibacterial, antifungal, and antioxidant activities [17,33,35–37].

Eight Malaysian banana cultivars showed a total phenolic content of 20.47 mg gallic acid equivalents (GAE)/100 g [6]. In 15 bananas cultivars that were grown in Viçosa, Minas Gerais, Brazil, the total phenolic content of the unripe peels ranged from 29.02 to 61.00 mg GAE/100 g and for ripe were between 60.39 to 115.70 mg GAE/100 g [38]. In addition, the total phenolic content was in an average of 88.31 mg tannic acid equivalents (TAE)/100 g peel (dry basis, d.b.) *M. paradisiaca* [39]. The tannin content was found to be 5800 mg TAE/100 g peel (d.b.) at the ripening stage and 1130 mg TAE/100 g peel (d.b.) at the maturation stage [40]. The flavonoid content that was found in the peel extract was 196 mg/g quercetin equivalent [41].

In the present study, rutin was identified with a high amount (973.08 mg/100 g DE), and previously Banana peel extract has been reported to contain naringinin, a flavanone glycoside, and rutin, a flavonol glycoside [42]. Other compounds, such as lutein, α- and β-carotene, auroxanthin, violaxanthin, neoxanthin, β-cryptoxanthin, isolutein, and α-cryptoxanthin have been identified in peel extracts [43]. Phenolic compounds, like ferulic acid (0.38%) and caffeic acid (0.06%), were identified in banana peel extract while using ultra-performance liquid chromatography with electrospray ionization (UHPLC–ESI [-]) [44].

Flavonoid compounds have been identified in high amounts, and one previous study reported that plantain peel flour had a total phenol level of 7.71 mg GAE/g, mainly comprising flavonoid type [8]. Banana peels were reported to contain various phenolic compounds, comprising catecholamines, flavanones, flavonols, and tocopherols [45].

The application of dihydroquercetin that was isolated from barley suppressed the growth of *Fusarium* spp. [46]. The flavonoid compound naringenin and its derivatives displayed both antifungal and antibacterial activities [47], which was found in the studied methanoilc extract in peels with an amount of 8.47 mg/100 g DE. The peel extract inhibited the growth of *Aspergillus niger*, *A. oryzae*, and *Rhizopus stolonifer* at a concentration of 1.0 mg/mL [48].

The methanol extract of *M. paradisiaca* peels showed a greater antibacterial activity than that of ethanol, water, and chloroform extracts against the human pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi* [1].

Peel extracts from three varieties of banana in powder and ash showed the presence of some phytochemicals, such as phenols, terpenoids, and saponins, and they exhibited antifungal activity against *A. niger*, but did not inhibit the growth of *A. flavus* or *Penicillium* spp. [49].

GA had observed potential antifungal activity against four studied yeast of *Candida* spps. [50]. Among dihydrokaempferol 3-O-β-glucopyranoside, dihydrokaempferol, GA, and ellagic acid that were identified from the EtOAc fraction of *Cochlospermum regium* roots, GA observed considerable antimicrobial effects against different bacterial and fungal species [51,52]. Ferulic (FA) and GA had antimicrobial activity against some pathogenic bacteria according to measured minimum inhibitory concentration MIC values [53]. The GA showed good antifungal activity against different strains of *Candida* [54,55].

Irreversible changes in membrane properties, such as extra/intra cellular permeability, decrease of negative surface charge, and physicochemical properties, as well as the local occurrence of rupture or pore formation in the cell membranes was found as FA and GA tested against the pathogenic bacteria [53].

Recently, wood that was treated with the flower extract of *Acacia saligna* showed the significant inhibition of *P. chrysogenum* mycelial growth, which could be related to the presence of benzoic acid, *o*-coumaric acid, naringenin, quercetin, and kaempferol [33]. Methanol extract from *Muscari aucheri* (flower + peduncle) with high rutin content found to be toxic (100%) against *F. oxysporum f.* sp. *cucumerinum*, *Alternaria solani*, *Verticillium dahliane*, *R. solani*, and *Botrytis cinerea* at 10 and 20 mg/mL doses [56]. Naringenin-7-O-b-D-glucopyranoside and rutin with four flavonoids compounds that were

identified in *Galium fissurense*, *Viscum album*, and *Cirsium hypoleucum* showed strong antimicrobial activities [47]. Additionally, rutin that was isolated from *Polygala paniculata* showed potential antifungal activity against *Cryptococcus gattii* and *Sporothrix schenckii* [57]. The presence of some flavonoid compounds, including myricetin, naringin, and rutin in *Phaleria macrocarpa* fruit were responsible for the antimicrobial activity [58,59]. The mechanisms of action of phenolic and flavonoid compounds were found to be the inhibition of cytoplasmic membrane function, nucleic acid synthesis, and energy metabolisms [58].

5. Conclusions

Mthanoilc extract of *M. paradisiaca* peels showed potential wood-biofungicide against the growth of *F. culmorum* and *R. solani*, and as a bactericide against *A. tumefaciens*, which could be considered a wood natural preservative during handling or in service. Using HPLC to analyze the phenolic and flavonoid compounds; gallic acid, catechol, ferulic acid, ellagic acid, *o*-coumaric acid, salicylic acid, cinnamic acid, rutin, myricetin, and naringenin were identified. Furthermore, the possible biological activities could be related to the presence of gallic acid, myricetin, and rutin in high amounts.

Author Contributions: S.I.B., M.E.-H., M.Z.M.S., M.K.O. and H.M.A. designed the experiments, conducted laboratory analyses, interpreted the results and wrote the manuscript; S.A.A., I.A.A., and S.M.A.-G. contributed reagents and materials, and J.C.M. with A.Z.M.S. revised the article for technical merits and final revision.

Funding: This research was funded by Dean of Scientific Research, King Saud University through the research group project number PRG-1439-63.

Acknowledgments: We extend our appreciation to the Dean of Scientific Research, King Saud University, for funding the work through the research group project number PRG-1439-63. The authors also thank the Deanship of Scientific Research and RSSU at King Saud University for their technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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