

Bioactivity of Selected Phenolic Acids and Hexane Extracts from *Bougainvillea spectabilis* and *Citharexylum spinosum* on the Growth of *Pectobacterium carotovorum* and *Dickeya solani* Bacteria: An Opportunity to Save the Environment

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Keywords: *Citharexylum* extract, *Bougainvillea*, *Dickeya*, *Pectobacterium*, ecofriendly environmental agents, phenolic acids

Abstract:

Phenolic acids and natural extracts, as ecofriendly environmental agents, can be used as bio bactericides against the growth of plant pathogenic bacteria. In this study, isolation trails from infected potato tubers and stems that showed soft rot symptoms in fields revealed two soft rot bacterial isolates and were initially identified through morphological, physiological, and pathogenicity tests. The molecular characterization of these isolates via PCR, based on the 16S rRNA region, was carried out by an analysis of the DNA sequence via BLAST and Genbank, and showed that the soft rot bacterial isolates belong to *Pectobacterium carotovorum* subsp. *carotovorum* (PCC1) and *Dickeya solani* (Ds1). The in vitro results of the tested phenolic acids against the cultured bacterial isolates proved that concentrations of 800, 1600, and 3200 µg/mL were the most effective. Ferulic acid was the potent suppressive phenolic acid tested against the Ds1 isolate, with an inhibition zone ranging from 6.00 to 25.75 mm at different concentrations (25-3200 µg/mL), but had no effect until reaching a concentration of 100 µg/mL in the PCC1 isolate, followed by tannic acid, which ranged from 7.00 to 25.50 mm. On the other hand, tannic acid resulted in a significant decrease in the growth rate of the PCC1 isolate with a mean of 9.11 mm. Chlorogenic acid was not as effective as the rest of the phenolic acids compared with the control. The n-hexane oily extract (HeOE) from *Bougainvillea spectabilis* bark showed the highest activity against PCC1 and Ds1, with inhibition zone values of 12 and 12.33 mm, respectively, at a concentration of 4000 µg/mL; while the HeOE from *Citharexylum spinosum* wood showed less activity. In the GC/MS analysis, nonanal, an oily liquid compound, was found at a percentage of 38.28%, followed by cis-2-nonenal (9.75%), which are the main compounds in *B. spectabilis* bark HeOE, and 2-undecenal (22.39%), trans-2-decenal (18.74%), and oleic acid (10.85%) were found, which are the main compounds in *C. spinosum* wood HeOE. In conclusion, the phenolic acids and plant HeOEs seem to raise the resistance of potato plants, improving their defense mechanisms against soft rot bacterial pathogens.

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Article

Bioactivity of Selected Phenolic Acids and Hexane Extracts from *Bougainvillea spectabilis* and *Citharexylum spinosum* on the Growth of *Pectobacterium carotovorum* and *Dickeya solani* Bacteria: An Opportunity to Save the Environment

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Abstract: Phenolic acids and natural extracts, as ecofriendly environmental agents, can be used as bio bactericides against the growth of plant pathogenic bacteria. In this study, isolation trails from infected potato tubers and stems that showed soft rot symptoms in fields revealed two soft rot bacterial isolates and were initially identified through morphological, physiological, and pathogenicity tests. The molecular characterization of these isolates via PCR, based on the 16S rRNA region, was carried out by an analysis of the DNA sequence via BLAST and Genbank, and showed that the soft rot bacterial isolates belong to *Pectobacterium carotovorum* subsp. *carotovorum* (PCC1) and *Dickeya solani* (Ds1). The *in vitro* results of the tested phenolic acids against the cultured bacterial isolates proved that concentrations of 800, 1600, and 3200 µg/mL were the most effective. Ferulic acid was the potent suppressive phenolic acid tested against the Ds1 isolate, with an inhibition zone ranging from 6.00 to 25.75 mm at different concentrations (25–3200 µg/mL), but had no effect until reaching a concentration of 100 µg/mL in the PCC1 isolate, followed by tannic acid, which ranged from 7.00 to 25.50 mm. On the other hand, tannic acid resulted in a significant decrease in the growth rate of the PCC1 isolate with a mean of 9.11 mm. Chlorogenic acid was not as effective as the rest of the phenolic acids compared with the control. The *n*-hexane oily extract (HeOE) from *Bougainvillea spectabilis* bark showed the highest activity against PCC1 and Ds1, with inhibition zone values of 12 and 12.33 mm, respectively, at a concentration of 4000 µg/mL; while the HeOE from *Citharexylum spinosum* wood showed less activity. In the GC/MS analysis, nonanal, an oily liquid compound, was found at a percentage of 38.28%, followed by *cis*-2-nonenal (9.75%), which are the main compounds in *B. spectabilis* bark HeOE, and 2-undecenal (22.39%), *trans*-2-decenal (18.74%), and oleic acid (10.85%) were found, which are the main compounds in *C. spinosum* wood HeOE. In conclusion, the phenolic acids and plant HeOEs seem to raise the resistance of potato plants, improving their defense mechanisms against soft rot bacterial pathogens.

Keywords: *Pectobacterium*; *Dickeya*; *Bougainvillea*; *Citharexylum* extract; ecofriendly environmental agents; phenolic acids

1. Introduction

Potato (*Solanum tuberosum* L.) is the world's fourth most consumed crop, with an estimated 374 million tons of production worldwide, obtained from nearly 17,623,660 hectares [1]. Potato is rated as one of Egypt's most significant vegetable crops, with a production total of 4,325,478 tons from around 163,939 hectares, making Egypt the second largest potato producer after Algeria. The main reasons for soft rot and blackleg disease in potatoes in warmer climates are *Pectobacterium carotovorum* subsp. *carotovorum* and *P. atrosepticum* [2,3], whereas, in Brazil and South Africa, the main causative agent for blackleg disease is *P. carotovorum* subsp. *brasiliensis* [4,5]. In early studies, *Erwinia chrysanthemi* was recognized as a causative agent of potato stem rot disease—recently reclassified as *Dickeya* spp. [6]. In Egypt, the main agents causing soft rot and blackleg disease are *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum* [7–10], and *Dickeya solani*, *P. carotovorum* subsp. *brasiliensis* [4,11,12]. Potatoes with soft rot cause massive losses of over 40% to 80% as a result of weather factors [13,14].

In the pathogenicity tests [15], 24 potato cultivars were tested for their susceptibility to soft rot caused by *P. atrosepticum* using a tuber slice test. The symptoms of soft rot on potato tuber, carrot, and sweet potato, as well as the fruits of eggplant and pepper, appear one to three days after inoculation with soft rot bacteria [16,17]. *D. solani* caused a greater loss of carrot tissue, higher than *P. carotovorum* subsp. *carotovorum* [18].

Chlorogenic, caffeic, and protocatechuic acids are the main phenolic acids in potato peels, while the mild phenolic acids are gallic, ferulic, and *p*-coumaric acids. The phenolic levels found in potato peels are significantly greater than in the potato flesh [19,20]. Phenolic acids are the first defense for potato tubers against *Pectobacteria* infection during wound healing, as they promote the inhibition of proteolytic activity or bactericide action [20–25].

Bougainvillea spectabilis (Bougainvillea), a popular woody shrub, grown in tropical and sub-tropical regions, has certain phytochemicals, such as saponins, quinones, flavonoids, triterpenoids, phenols, sterols, glycosides, furanoids, tannins, and small amounts of sugars [26–29]. *B. spectabilis* leaves contain d-pinitol (3-O-methylchiroinositol) [30]. Ethanolic and methanolic extracts from *B. spectabilis* leaves show a good antimicrobial effect against Gram-positive and -negative bacteria, and could replace the use of antibiotics [31].

Citharexylum spinosum (*C. quadrangulare* or *C. fruticosum*) belongs to the Verbenaceae family. *Citharexylum* species have shown good biological activities, such as antioxidant, nephroprotector, anti-inflammatory, gastroprotector, hypoglycemic, antipyretic, and antibacterial activities [32–35]. Carotenoids, iridoids, flavonoids, terpenoids, alkaloids, and saponines, which were isolated and identified from the extracts of *Citharexylum* species [32,36–38].

The objectives of the present study were to isolate and identify potato soft rot bacteria through classical and molecular tests, in order to determine the sensitivity of soft rot bacteria *Pectobacterium carotovorum* subsp. *carotovorum* (PCC1) and *Dickeya solani* (Ds1) toward some phenolic acids and plant extracts from *B. spectabilis* bark and *C. spinosum* wood.

2. Materials and Methods

2.1. Isolation and Conventional Identification of the Soft Rot Bacteria

Potato tubers showing soft rot and stems exhibiting blackleg symptoms were collected from different localities at El-Behira Governorate, Egypt (Table 1), and a bacterial pathogens isolation procedure was performed [39]. The morphological and biochemical characteristics tests were applied on the obtained soft rot bacterial isolates, and included cell shape, Gram staining, motility, anaerobic growth, growth at 36 °C, gelatin liquefaction, indole formation, nitrate reduction, hydrolysis of starch, lipolytic activity, mucoid growth, H₂S production from cysteine, reducing substance from sucrose, acetoin production, urease production, oxidase, growth in 5% NaCl, and sensitivity to the antibiotic erythromycin [40]. The bacterial isolates were molecularly identified through 16S rRNA gene sequencing, according to Ashmawy et al. [16].

2.2. Molecular Identification Through the 16S rRNA Gene

After bacterial DNA isolation by CTAB method [16], a full length of the 16S rRNA gene (1550-bp) was amplified for the two bacterial isolates using primers—P0 as the forward (5'-GAAGAGTTTGATCCTGGCTCAG-3') and P6 as the reverse (5'-CTACGGCTACCTTGTGTTACGA-3'). PCR amplification was performed in a total volume of 50 μ L, containing 25 μ L of master mix (enzymocis, korea), 2 μ L of each P0 or P6 primer (10 pmol) with final concentration 0.1–0.5 μ M of each primer, 2 μ L (50 ng/ μ L) of bacterial genomic DNA, and molecular grade water was added until the volume reached 50 μ L. The PCR reaction was carried out as follows: 1 cycle at 95 °C (5 min) for initial denaturation, and 35 cycles (denaturation for 45 s at 95 °C, annealing for 60 s at 50 °C, and elongation for 120 s at 72 °C) for the final extension, for 7 min at 72 °C. PCR amplicons were visualized by an ultra-violet (UV) transilluminator [16].

Sequencing of 16S rRNA Gene and BLASTn

The amplified amplicons of the 16S rRNA gene were purified and sequenced by a BigDye[®] Terminator v1.1 Cycle Sequencing Kit (ThermoFisher SCIENTIFIC, Waltham, MA, USA) and analyzed by 3130 Genetic Analyzer (Macrogen Co., Seoul, Korea). Alignment of the nucleotide sequences was performed with MSA CLUSTAL (Omega <https://www.ebi.ac.uk/Tools/msa/>) [16]. BLASTn was used for the nucleotide sequences comparisons on the GenBank website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) [41,42].

2.3. Plant Material and Pathogenicity Test

Potato tuber cultivar “Diamont” was obtained and examined for its ability to exhibit the soft rot symptoms, using the two bacterial isolates as cited by Manzira [14], and the disease severity index was estimated as $PDI = [(A - B) / A] * 100$. Here, PDI is the percentage of disease severity index, A is the tuber weight with rotting, and B is the tuber weight without rotting [43].

2.4. Source of Phenolic Acids, Extraction Method of Plant Parts Used, and GC/MS Analysis

The phenolic compounds of caffeic, tannic, *p*-coumaric, protocatechuic, chlorogenic, and ferulic acid were purchased from Sigma-Aldrich (Merck). Samples of *Bougainvillea spectabilis* Willd. and *Citharexylum spinosum* L. plants were collected from Alexandria, Egypt, during September, 2018 and authenticated by Dr. Mohamed Z.M. Salem, Department of Forestry and Wood Technology, Alexandria University, Alexandria, Egypt (Voucher number Zidan0059, and Zidan0060, respectively). The extracts from *B. spectabilis* bark and *C. spinosum* wood were prepared by soaking 50 g of each part of the plant—in the form of powdered material after air-drying—in *n*-hexane (150 mL) for 6 h under shaking, after which the extract was concentrated in a vacuum using a rotary evaporator.

2.5. Influence of Some Phenolic Acids and Plant Extracts on Bacterial Growth

The two bacterial isolates were tested against 0, 25, 50, 100, 200, 400, 800, 1600, and 3200 μ g/mL concentrations of caffeic, tannic, *p*-coumaric, protocatechuic, chlorogenic, and ferulic acids using agar-well diffusion method in a nutrient agar (NA) medium. After 48 h of incubation, the inhibition zone (mm) was measured, and the assays were replicated three times and the experiments conducted twice [44]. The extracts were prepared at concentrations of 125, 250, 500, 1000, 2000, and 4000 μ g/mL.

The chemical compositions of the *n*-hexane oily extracts (HeOEs) from *B. spectabilis* bark and *C. spinosum* wood were analyzed using a Focus GC-DSQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column of TG-5MS (30 m \times 0.25 mm \times 0.25 μ m film thickness). The temperature programs and column separation conditions can be found in previous work [45]. Identification of the compounds was done by a comparison of their retention times, as well as the MS reported from the WILEY 09 and NIST 11 mass spectral databases [46]. The values of the standard

index (SI) and reverse standard index (RSI) were also reported in order to confirm that all of the spectra were appended to the library [47,48].

2.6. Statistical Analysis

The data were analyzed statistically with a two-way analysis of variance (ANOVA) using SAS software (SAS Institute, NC, USA) [49]. The two factors that analyzed were phenolic and extracts, as well as their respective concentrations. The means of the treatments were compared with control treatment, according to the Duncan's Multiple Range Test at a 0.05 level of probability.

3. Results

3.1. Isolation Trails of the Causal Bacterial Pathogens

The isolation trails of the soft rot and blackleg symptoms (Figure 1) collected from the El-Nubaria and Wadi Elnatron regions, Egypt, revealed two bacterial isolates PCC1 and Ds1 which belonging to *Pectobacterium* and *Dickeya* genera, respectively (Table 1).



Figure 1. Natural infection of potato tubers with soft rot and blackleg symptoms: **(left)** *Pectobacterium carotovorum* subsp. *carotovorum* (PCC1) and **(right)** *Dickeya solani* (Ds1).

Table 1. Origin and disease index of soft rot and blackleg bacterial isolates.

Bacterial Genera	Isolates Code	Potato Part	Cultivar	Origin	Disease Severity Index \pm SD
<i>Pectobacterium</i>	PCC1	Tuber	Roseta	El-Nubaria, El-Behira, Egypt	86.04 \pm 0.97
<i>Dickeya</i>	Ds1	Stem	Hermes	Wadi elnatron, El-Behira, Egypt	71.62 \pm 0.53
Control					0.00 \pm 0.00

3.2. Phenotypic and Molecular Identification of the Soft Rot Bacteria

Based on the morphological, biochemical, and physiological characteristics of the isolated soft rot bacteria, the bacterial isolates were identified as *Pectobacterium carotovorum* subsp. *carotovorum* (PCC1) and *Dickeya solani* (Ds1) (Table 2). The identification of the isolates PCC1 and Ds1 was confirmed using the 16S rDNA sequences analysis, and was deposited in the GenBank database under accession numbers MN598002 and MN598003, respectively.

Table 2. Morphological traits and physiological and biochemical reactions of *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya solani* isolates.

Characteristics	Bacterial Isolates	
	<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	<i>Dickeya solani</i>
Shape (rods)	+	+
Gram staining	–	–
Motility	+	+
Anaerobic growth	+	+
Potato soft rot	+	+
Growth at 37 °C	+	+
Gelatin liquefaction	+	+
Mucoid growth	+	+
Kovac’s oxidase	–	–
H ₂ S from cysteine	+	+
Indole production	–	+
R. substance from sucrose	–	–
Urease production	–	–
Growth in 5% NaCl	+	–
Sensitivity to erythromycin	–	+
Phosphatase	–	+
Malonate utilization	–	+
Starch hydrolysis	+	+
Glucose	a	a
α-methyl glucoside	–	–
Maltose	–	a
Lactose	a	a
L-Arabinose	a	a
Dulcitol	a	a
Manitol	a	a
Trehalose	a	–

Note: “+” = positive reaction; “–” = negative reaction; a = acid.

3.3. Pathogenicity Tests

The two tested bacterial isolates were pathogenic and produced soft rot symptoms on potato tubers. The PCC1 isolate showed a high disease index (86.04%), while the disease index of the isolate Ds1 was 71.62% (Table 1).

3.4. Influence of Some Phenolic Acids and Plant Oily Extracts on Growth of PCC1 and Ds1 Isolates

The data presented in Table 3 show the highly significant effects of the tested phenolic acids/oily extracts and their concentrations against the growth of PCC1 and Ds1. Table 4 shows that the different concentrations of the tested phenolic acids or the n-hexane oily extracts (HeOEs) from *Bougainvillea spectabilis* bark and *Citharexylum spinosum* wood caused different degrees of growth inhibition on the PCC1 and Ds1 isolates. It is evident that ferulic acid was the most suppressive to Ds1 isolate growth, with an inhibition zone (IZ) that ranged from 6 to 25.75 mm but had no effect on the PCC1 isolate growth until reaching a concentration of 100 µg/mL. On the other hand, tannic acid application decreased the growth rate of the PCC1 isolate with a mean of 9.11 mm. Finally, chlorogenic acid was less effective than all of the other phenolic acids used compared with the control. Significant differences were found among all phenolics at concentrations of 400 and 800 µg/mL. On the other hand, phenolic acid concentrations of 25 and 50 µg/mL had no noticeable effect on the two isolates, except for ferulic acid. Overall, the PCC1 isolate was more tolerant to all of the phenolic acids than the Ds1 isolate, and the applied concentrations of 800, 1600, and 3200 µg/mL were the most effective at inhibiting the two isolates.

Table 3. Analysis of variance (ANOVA) for the significance effects of phenolic/extract, concentration, and their interaction against the growth of *P. carotovorum* and *D. solani*.

Source of Variance	DF	Type III SS	Mean Square	F Value	Pr > F
PCC1					
Concentrations (A)	12	6604.123	550.343	1651.91	<0.0001
Phenolic/extract (B)	6	269.392	44.898	134.77	<0.0001
A × B	40	393.7006	9.842	29.54	<0.0001
Ds1					
A	12	8346.289	695.524	5894.27	<0.0001
B	6	1843.256	307.209	2603.47	<0.0001
A × B	40	939.368	23.484	199.02	<0.0001

Table 4. Effect of phenolic acids/oily extracts at various concentrations against the growth of *P. carotovorum* subsp. *carotovorum* (PCC1) and *D. solani* (Ds1).

Phenolic Acids/Extracts	Concentrations µg/mL	Inhibition Zone Diameter (mm) ± SE	
		PCC1	Ds1
Caffeic acid	25	0.00	0.00
	50	0.00	0.00
	100	7 ± 0.00	0.00
	200	7 ± 0.00	0.00
	400	8.5 ± 0.28	6 ± 0.00
	800	13.75 ± 0.14	7.75 ± 0.14
	1600	18 ± 0.00	18.75 ± 0.14
	3200	20.7 ± 0.46	22.75 ± 0.72
Tannic acid	25	0.00	0.00
	50	0.00	0.00
	100	7 ± 0.00	7 ± 0.00
	200	8.75 ± 0.14	11.75 ± 0.14
	400	11.5 ± 0.28	15.25 ± 0.14
	800	15.25 ± 0.14	17.25 ± 0.14
	1600	17.25 ± 0.14	21.5 ± 0.28
	3200	22.25 ± 0.14	25.5 ± 0.00
<i>p</i> -Coumaric acid	25	0.00	0.00
	50	0.00	0.00
	100	0.00	8 ± 0.00
	200	0.00	9 ± 0.00
	400	7 ± 0.00	9 ± 0.00
	800	10.25 ± 0.14	14 ± 0.00
	1600	13 ± 0.00	17.5 ± 0.28
	3200	18 ± 0.28	20.25 ± 0.14
Protocatechuic acid	25	0.00	0.00
	50	0.00	0.00
	100	7 ± 0.00	0.00
	200	7 ± 0.00	6 ± 0.00
	400	10.5 ± 0.28	8 ± 0.00
	800	11.87 ± 0.36	8.75 ± 0.14
	1600	12 ± 0.00	11 ± 0.00
	3200	14.75 ± 0.14	14.5 ± 0.28

Table 4. Cont.

Phenolic Acids/Extracts	Concentrations $\mu\text{g/mL}$	Inhibition Zone Diameter (mm) \pm SE	
		PCC1	Ds1
Chlorogenic acid	25	0.00	0.00
	50	0.00	0.00
	100	0.00	0.00
	200	6 \pm 0.00	11.5 \pm 0.00
	400	10 \pm 0.00	15.5 \pm 0.00
	800	13.5 \pm 0.28	22 \pm 0.28
	1600	18.5 \pm 0.28	25.5 \pm 0.00
	3200	19.25 \pm 1.01	25.25 \pm 0.43
Ferulic acid	25	0.00	6 \pm 0.00
	50	0.00	9 \pm 0.00
	100	6.5 \pm 0.28	11.5 \pm 0.28
	200	7 \pm 0.00	14.75 \pm 0.14
	400	8.5 \pm 0.00	18.5 \pm 0.00
	800	12.25 \pm 0.14	22.5 \pm 0.00
	1600	17.75 \pm 0.14	24.25 \pm 0.14
	3200	21.5 \pm 0.57	25.75 \pm 0.14
<i>Bougainvillea spectabilis</i> bark	125	6.66 \pm 0.88	6.83 \pm 0.16
	250	7.33 \pm 0.66	7.16 \pm 0.16
	500	7.33 \pm 0.33	9.33 \pm 0.46
	1000	9 \pm 0.57	10 \pm 0.33
	2000	9.66 \pm 0.33	11 \pm 0.22
	4000	12 \pm 0.57	12.33 \pm 0.33
<i>Citharexylum spinosum</i> wood	125	6.33 \pm 0.88	6.16 \pm 0.44
	250	6.66 \pm 0.66	6.5 \pm 0.28
	500	6.66 \pm 0.66	7.5 \pm 0.28
	1000	7 \pm 0.57	7.83 \pm 0.16
	2000	8.66 \pm 0.33	8.33 \pm 0.33
	4000	10 \pm 0.57	8.5 \pm 0.28
Control	0	0.00	0.00
<i>p</i> -value		**	**

Note: SE = standard error; ** = highly significance at a 0.01 level of probability.

Additionally, from Table 4, the *n*-hexane oily extracts (HeOEs) from *B. spectabilis* bark and *C. spinosum* wood showed that with increasing the HeOE concentration, the IZ observed against the growth of PCC1 and Ds1 was increased. The highest IZ (12 mm) against PCC1 was observed for *B. spectabilis* bark HeOE applied at a concentration of 4000 $\mu\text{g/mL}$, followed by the same HeOE with an IZ of 9.66 mm at a concentration of 2000 $\mu\text{g/mL}$. Furthermore, *B. spectabilis* bark HeOE at 4000, 2000, and 1000 $\mu\text{g/mL}$ showed the highest IZs against the growth of Ds1, with values of 12.33, 11, and 10.33 mm, respectively. Furthermore, *C. spinosum* HeOE showed an IZ value of 10 mm against the growth of PCC1 at 4000 $\mu\text{g/mL}$ level of concentration. Overall, the phenolic acids showed the highest activity against the growth of both of the bacteria, compared with the HeOEs.

3.5. Chemical Constituents of *B. spectabilis* Bark and *C. spinosum* Wood Oily Extracts

Table 5 presents the chemical composition of the *B. spectabilis* bark HeOE. The main dominant compounds were nonanal (38.28%), *cis*-2-nonenal (9.75%), octanal (8.16%), β -sitosterol (7.8%), 3-hydroxy-dodecanoic acid (6.9%), heptanal (4.03%), 8-oxabicyclo[5.1.0]octane (3.50%), (*E*)-2-octen-1-ol (2.68%), 1-decene (1.92%), (*E*)-2-decen-1-ol (1.84%), 9-oxabicyclo[6.1.0]nonan-4-ol (1.39%), and 1-chlorohexane (1.18%).

Table 5. Phytochemicals of *B. spectabilis* bark HeOE by GC/MS.

Compound	Value in the Extract (%)	SI ¹	RSI ²
Hex-2-ulosonic acid	0.49	659	718
1-Chlorohexane	1.18	675	683
5-heptyldihydro-2(3H)-furanone	0.57	710	725
2-Ethylpentane	0.53	707	873
Octane	0.54	816	877
Hexanal	0.72	773	808
2-Hexyl-cyclopropaneacetic acid	0.33	749	789
9-Oxabicyclo[6.1.0]nonan-4-ol	1.39	665	674
2-Undecanol	0.72	648	847
1-Hydroperoxyhexane	0.48	646	745
Heptanal	4.03	763	817
β -sitosterol	7.8	838	951
(E)-2-Decen-1-ol	1.84	681	686
8-Oxabicyclo[5.1.0]octane	3.50	692	745
Isopinocarveol	0.93	651	686
Octanal	8.16	814	832
8,11-Octadecadiynoic acid methyl ester	0.78	684	691
5-Isopropenyl-2-methyl-2-cyclohexen-1-ol	0.40	675	684
(E)-2-Octen-1-ol	2.68	770	823
<i>trans</i> -Pinocarveol	0.27	700	758
(Z)-2-Tridecenal	0.58	723	792
2-Hexyl-cyclopropaneacetic acid	0.20	710	754
1-Decene	1.92	737	741
Nonanal (Pelargonaldehyde)	38.28	896	912
Tetradecan-1-ol	0.30	691	693
13,16-Octadecadiynoic acid methyl ester	0.98	680	688
2-Phenylbutanal	0.94	676	680
<i>cis</i> -2-Nonenal	9.75	792	885
3-Hydroxy-dodecanoic acid	6.9	736	737

¹: SI = standard index; ²: RSI = reverse standard index.

The chemical compositions of the HeOE from *C. spinosum* wood are shown in Table 6. The abundant chemical constituents were 2-undecenal (22.39%), *trans*-2-decenal (18.74%), oleic acid (10.85%), nonanal (9.75%), 2-methylenecholestan-3-ol (6.01%), (Z)-2-tridecenal (4.03%), Z-(13,14-epoxy)tetradec-11-en-1-ol acetate (3.58%), 3-hydroxy-dodecanoic acid (3.34%), 9-hexadecenoic acid (2.3%), 1-dodecene (1.96%), (E)-2-nonenal (1.78%), octanal (1.72%), and 12,15-octadecadiynoic acid methyl ester (1.7%).

Table 6. Phytochemicals of *C. spinosum* wood HeOE by GC/MS.

Compound	Value in the Extract (%)	SI ¹	RSI ²
2,7-dimethyl-1-Octanol	0.32	721	787
1-Dodecene	1.96	763	763
2-Undecanol	1.39	848	172
Octanal	1.72	809	838
(Z)-2-Tridecenal	4.03	778	838
Nonanal	9.75	889	914
Hexadecanoic acid phenylmethyl ester	0.92	706	720
(E)-2-Nonenal	1.78	786	902
3-Hydroxy-dodecanoic acid	3.34	821	821
<i>trans</i> -2-Decenal	18.74	881	926
β -Hydroxydodecanoic acid	0.77	797	799
(E,E)-2,4-Dodecadienal	1.48	795	803
2-methylenecholestan-3-ol	6.01	749	868
2-Undecenal	22.39	899	926
1,2-15,16-Diepoxyhexadecane	1.21	798	807
1-acetyl-16-methoxy-aspidospermidin-17-ol	1.04	800	830
9-Hexadecenoic acid	2.3	806	811
12,15-Octadecadiynoic acid methyl ester	1.7	770	793
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	3.58	804	813
1-Heptatriacotanol	0.9	790	796
Oleic acid	10.85	857	859

¹: SI = standard index; ²: RSI = reverse standard index.

4. Discussion

Soft rot disease causes huge economic losses, estimated to be between 40% to 80% depending on climatic conditions, and *Pectobacterium carotovorum* subsp. *carotovorum* (PCC1) and *Dickeya solani* (Ds1) are the causal agents of soft rot disease in potato tubers in stores and in the field, where the early decay of mother tubers or seed tuber pieces may occur [13,14,50–52]. The pathological behavior of the isolated bacterial cultures, as well as their cultural, morphological, and physiological characters conform to those known for all soft rot bacteria. On the basis of the obtained data, we could identify these isolates as PCC1 and Ds1, in the same way as many other researchers have in previous works [4,11,12,40,53–55].

Nowadays, the major objective of modern Egyptian agriculture is to offer a strategy that would lead to minimizing the use of chemical pesticides, at the same time increasing the economic yield of crops. Therefore, much attention has been given to hinder the severity and spread of plant diseases, especially bacterial plant pathogens, by using all possible non-polluting methods of plant disease control. The objective of this research was to describe the tolerance of isolates PCC1 and Ds1 to phenolic acids. The findings in the present work showed that ferulic and tannic acids had a substantial inhibitory impact on the growth of Ds1 and PCC1 isolates. A mixture of caffeic and chlorogenic acids could prevent bacterial soft rot infection from occurring, and the major phenolic acids detected in the tuber peels that had soft rot antimicrobial effects were chlorogenic, caffeic, and ferulic acids [21,22].

Tannic acid inhibited the growth of certain bacterial strains [56], while tannic and gallic acids inhibited the growth and protease or pectatelyase enzyme activities of the soft rot isolate *D. solani* [23]. A more pronounced antimicrobial impact at different concentrations was found for tannic acid. The size difference and percentage of oH⁻ groups between ferulic and tannic acids can explain this varied response against soft rot bacterial pathogens [57]. Both phenolic acids can affect pathogen growth by contact with the produced protease and pectate lyase enzymes, the effective mechanism could be described as protein inhibitors by modifying their stability and losing cellular permeability, or by reducing the substrate availability or chelating the metal co-factor, as the tannic acid can fix the iron metal [58–63]. In this study, both isolates (PCC1 and Ds1) were growth inhibited by the examined polyphenols, and we suggest that the mode of action could interact and inactivate the enzyme active sites, which leads to precipitating the enzymatic proteins. This is in agreement with several authors who have talked about the mechanisms of tannic acid, polyphenol compounds, and their significant biological impacts, for example as bactericidal, antiviral, or fungal repressors [64,65].

Fatty acid and fatty alcohols, such as *n*-octacos-9-enoic acid and *n*-hentriacontanol, were isolated from *Bougainvillea spectabilis* roots [66]. Butyl formate, butyl acetate, methyl 2-methylbutanoate, methyl hexadecanoate, ethyl hexadecanoate, hexanal, heptanal, ethyl 3-hydroxy-hexanoate, and methyl linolenate were isolated from leaves and branches [67]. (*Z*)-2-hexenal, linalool, 2-heptadecanone, toluene, *O*-xylene, 2-furfural, terpinolene, terpinen-4-ol, and methyl salicylate were identified in the leaves and branches of *B. spectabilis* [67]. Compounds of bougainvinone A-M were isolated from stem bark of *B. spectabilis* [26], also, quercitrin as a flavonoid compound has been isolated from the stem bark [28]. Different solvent extractions, such as methanol, ethanol, water, chloroform, and ethyl acetate, were used to extract the chemical compounds from different parts of *B. spectabilis*, and have observed a good antibacterial activity [68–70]. *B. spectabilis* might be considered as a potential source of metabolites, which could be developed as precursors for antimicrobial and antioxidant drugs [71].

Citharexylum spinosum has been reported to have some biological isolated compounds, such as 5-deoxy pulchellose, 8-epiloganin, iridoid glucoside, lamiidoside, duranterectoside C, and the lignan glucoside [36,72]. Flower essential oil and extracts exhibited antibacterial and antioxidant activities [33,73,74]. At 8 µg/mL of concentrated methanol extract of *C. spinosum* wood, there was a potent inhibition against the growth of *P. variotii* seen [75]. The *B. spectabilis* extract was more effective than *C. spinosum* extract, and this may be because it contains aldehydes and huge amounts of volatile compounds, such as nonanal, which was found in the phytochemical analysis at a percentage of 38.28%. The biological activities of nonanal have only been reported in a few publications, as it significantly inhibits the mycelia growth of *P. cyclospium* [76].

The inhibitory effect of *B. spectabilis* extract could correlate with the concentration of nonanal *versus* *C. spinosum* wood extracts, and these results are the same as other reports [77–79]. Inhibition against *A. niger* and *P. sclerotigenum* growth was found with minimum inhibitory concentration (MIC) values of 250 µg/mL and 500 µg/mL, respectively [76]. Chloroform leaf extract from *C. spinosum* showed a weak activity against *P. carotovorum* subsp. *carotovorum*, *P. atrosepticum* and *D. solani*, [80]. The stem-bark ethyl acetate extract of *C. spinosum* showed the presence of vanillic acid [38]. *p*-coumaric acid, salicylic acid, and hispidulin were identified in the *Citharexylum* genus to have a good antimicrobial activity [81]. The *n*-butanol extract and essential oil (EO) of the cones of *Pinus halepensis* had a great antibacterial effect against the soft rot bacteria *D. solani* and *P. atrosepticum* [82].

Nonanal, the main oily compound found *B. spectabilis* bark HeOE, a saturated fatty aldehyde, arises from a reduction in the carboxy group of nonanoic acid. The unexplainable phenomena were not noted in nonanal alone, suggesting that aldehyde hydrocarbons are much more effective in managing postharvest diseases than alcohols and olefine [83]. Octanal and nonanal showed medium activity among the aldehyde constituents [84].

The prospective concepts underlying the antimicrobial activity of aldehyde and terpenes are not fully realized, but a number of possible strategies have been proposed. It is recognized that Gram-negative bacteria are more resistant than Gram-positive bacteria to EOs components [85,86]. Unsaturated aldehydes such as (*E*)-2-hexenal, (*E*)-2-octenal, and (*E*)-2-nonenal have been shown a noticeable activity against several fungal and bacterial isolates [87,88]. Thus, these aldehydes might be good compounds for playing a reserving role against human diseases caused by bacteria or as food preservatives, or might be a good alternative to other highly toxic disinfectants for hospital equipment. Recently, *Pinus halepensis* branch HeOE showed the presence of 2-undecenal, (*Z*)-2-decenal, nonanal, (*2E*)-2-decenal, and decadienal as main compounds, with a good antifungal activity against *B. oryzae* and *F. oxysporum* [89].

In the present study, *in vitro* antibacterial activity has encouraged us to assume that the potential antibacterial activity of nonanal, an essential compounds from hydrophobic oil, against *P. carotovorum* subsp. *carotovorum*, and *D. solani* could be closely correlated with the physiology of the membrane [90–92]. In addition, fatty acid methyl esters or aldehydes are able to penetrate the hydrophobic regions of the membranes, and the carboxyl groups pass through the cell membrane, causing the lowering of the internal pH and denaturing of proteins inside the cell [93–96].

The most bioactive molecules found naturally in plants are phenolics, such as tannins and lignans. The hydroxycinnamic (a) and the hydroxybenzoic (b) acids, are two main groups of phenolics; (a) group contains caffeic, ferulic and *p*-coumaric acids, but the (b) group contains gallic, protocatechic acids [97,98]. *p*-Coumaric acid is the stepping stone in synthesis process of caffeic, chlorogenic and ferulic acids, and these phenolics lead to have an antimicrobial and antiviral effects in different mode of actions as it could kill the fungal and bacterial cells by breakdowns and ruptures the plasma membrane [99–103]. In another study, the cinnamic acid proved to be effective in suppressing the virulent species of *Pectobacterium* spp. by blocks the quorum sensing molecules [22,104]. Several studies documented the strong antibacterial activity of the commercial form of caffeic, chlorogenic, and *p*-coumaric acids against multi bacterial isolates such as *E. coli*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Salmonella typhimurium*, with a minimum inhibitory concentration (MIC) values ranging from 8–1000 µg/mL [105–108]. While Wang et al. [109], confirmed the broad spectrum of antibacterial activity of the ferulic acid sourced from *Halimodendron halodendron* (Pall.) plant material towards the plant bacterial strains *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *lachrymans*, *Xanthomonas campestris* pv. *vesicatoria* [109].

5. Conclusions

In the present study, isolates from *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya solani* were conventionally and molecularly identified, and were proven to be pathogenic and cause potato soft rot. Oily extracts of *Bougainvillea spectabilis* bark (Ca. 4000, 2000, and 1000 µg/mL) at phenolic acid

concentrations of 800, 1600, and 3200 µg/mL were the most effective against bacterial isolate growth. Our present study suggests that phenolics and plant extracts might be used as bactericides to fight against soft rot bacterial diseases.

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References

1. FAO STAT. Agriculture Data Source. 2017. Available online: <http://faostat.fao.org/default.aspx> (accessed on 17 May 2017).
2. Avrova, A.O.; Hyman, L.J.; Toth, R.L.; Toth, I.K. Application of amplified fragment length polymorphism fingerprinting for taxonomy and identification of the soft rot bacteria *Erwinia carotovora* and *Erwinia solani*. *App. Environ. Microbiol.* **2002**, *68*, 1499–1508. [[CrossRef](#)] [[PubMed](#)]
3. Pérombelon, M.C.M. Potato diseases caused by soft rot erwinias: An overview of pathogenesis. *Plant Pathol.* **2002**, *51*, 1–12. [[CrossRef](#)]
4. Duarte, V.; De Boer, S.H.; Ward, L.J.; De Oliveira, A.M.R. Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. *J. App. Microbiol.* **2004**, *96*, 535–545. [[CrossRef](#)] [[PubMed](#)]
5. Van der Merwe, J.J.; Coutinho, T.A.; Korsten, L.; van der Waals, J.E. *Pectobacterium carotovorum* subsp. *brasiliensis* causing blackleg on potatoes in South Africa. *Eur. J. Plant Pathol.* **2010**, *126*, 175–185. [[CrossRef](#)]
6. Zhang, Y.; Fan, Q.; Loria, R. A re-evaluation of the taxonomy of phytopathogenic genera *Dickeya* and *Pectobacterium* using whole-genome sequencing data. *Syst. Appl. Microbiol.* **2016**, *39*, 252–259. [[CrossRef](#)] [[PubMed](#)]
7. Ashmawy, N.A.; Behiry, S.I.; Ali, H.M.; Salem, M.Z.M. Evaluation of *Tecomastans* and *Callistemon viminalis* extracts against Potato soft rot bacteria *in vitro*. *J. Pure Appl. Microbiol.* **2014**, *8*, 667–673.
8. Behiry, S.I.; Okla, M.K.; Alamri, S.A.; EL-Hefny, M.; Salem, M.Z.M.; Alaraidh, I.A.; Ali, H.M.; Al-Ghtani, S.M.; Monroy, J.C.; Salem, A.Z.M. Antifungal and antibacterial activities of *Musa paradisiaca* L. peel extract: HPLC analysis of phenolic and flavonoid contents. *Processes* **2019**, *7*, 215. [[CrossRef](#)]
9. Okla, M.K.; Alamri, S.A.; Salem, M.Z.M.; Ali, H.M.; Behiry, S.I.; Nasser, R.A.; Soufan, W. Yield, Phytochemical Constituents, and Antibacterial Activity of Essential Oils from the Leaves/Twigs, Branches, Branch Wood, and Branch Bark of Sour Orange (*Citrus aurantium* L.). *Processes* **2019**, *7*, 363. [[CrossRef](#)]
10. Al-Huqail, A.A.; Behiry, S.I.; Salem, M.Z.M.; Ali, H.M.; Siddiqui, M.H.; Salem, A.Z.M. Antifungal, Antibacterial, and Antioxidant Activities of *Acacia Saligna* (Labill.) H. L. Wendl. Flower Extract: HPLC Analysis of Phenolic and Flavonoid Compounds. *Molecules* **2019**, *24*, 700. [[CrossRef](#)]
11. Laurila, J.; Ahola, V.; Lehtinen, A.; Joutsjoki, T.; Hannukkala, A.; Rahkonen, A.; Pirhonen, M. Characterization of *Dickeya* strains isolated from potato and river water samples in Finland. *Eur. J. Plant Pathol.* **2008**, *122*, 213–225. [[CrossRef](#)]
12. Cating, R.A.; Palmateer, A.J. First report of a bacterial soft rot on *Tolumnia* Orchids caused by a *Dickeya* sp. in the United States. *Plant Dis.* **2009**, *93*, 1354. [[CrossRef](#)] [[PubMed](#)]
13. Ngadze, E.; Icishahayo, D. Survey: To assess the distribution and impact of potato blackleg and soft rot diseases in Zimbabwe. *IOSR J. Agric. Vet. Sci.* **2014**, *7*, 126–132. [[CrossRef](#)]
14. Ngadze, E.; Brady, C.L.; Coutinho, T.A.; van der Waals, J.E. Pectinolytic bacteria associated with potato soft rot and blackleg in South Africa and Zimbabwe. *Eur. J. Plant Pathol.* **2012**, *134*, 533–549. [[CrossRef](#)]
15. Lapwood, D.H.; Read, P.J. A simplified slice method for assessing tuber susceptibility of potato cultivars to *Erwinia carotovora* subsp. *atroseptica*. *Plant Pathol.* **1985**, *34*, 284–286. [[CrossRef](#)]

16. Ashmawy, N.A.; El-Bebany, A.F.; Shams, A.H.; Shoeib, A.A. Identification and differentiation of soft rot and blackleg bacteria from potato using nested and multiplex PCR. *J. Plant Dis Prot.* **2020**, *127*, 141–153. [[CrossRef](#)]
17. Saleh, O.I.; Huang, J.S. Bacterial soft rot disease of tomato fruits in Florida USA: Identification, response of some American and Egyptian cultivars of solanaceous plants and chemical control. *Assiut J. Agric. Sci.* **1997**, *28*, 11–26.
18. Farrar, J.J.; Nunez, J.J.; Davis, R.M. Influence of soil saturation and temperature on *Erwinia solani* soft rot of carrot. *Plant Dis.* **2000**, *84*, 665–668. [[CrossRef](#)]
19. Mohdaly, A.; Sarhan, M.; Mahmoud, A.; Ramadan, M.; Smetanska, I. Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection. *Food Chem.* **2010**, *123*, 1019–1026. [[CrossRef](#)]
20. Deusser, H.; Guignard, C.; Hoffmann, L.; Evers, D. Polyphenol and glycoalkaloid contents in potato cultivars grown in Luxembourg. *Food Chem.* **2012**, *135*, 2814–2824. [[CrossRef](#)]
21. Kumar, A.; Pundhir, V.S.; Gupta, K.C. The role of phenols in potato tuber resistance against soft rot by *Erwinia carotovora* ssp. *carotovora*. *Potato Res.* **1991**, *34*, 9–16. [[CrossRef](#)]
22. Joshi, J.R.; Burdman, S.; Lipsky, A.; Yariv, S.; Yedidia, I. Plant phenolic acids affect the virulence of *Pectobacterium aroidearum* and *P. carotovorum* ssp. *brasiliense* via quorum sensing regulation. *Mol. Plant Pathol.* **2015**, *17*, 487–500. [[CrossRef](#)]
23. Yahiaoui-Zaidi, R.; Zaidi, F.; Ait Bessai, A. Influence of gallic and tannic acids on enzymatic activity and growth of *Pectobacterium solani* (*Dickeya solani* ssp. *solani*). *Afr. J. Biotechnol.* **2008**, *7*, 482–486.
24. Lagonenko, L.; Lagonenko, A.; Evtushenkov, A. Impact of salicylic acid on biofilm formation by plant pathogenic bacteria. *J. Biol. Earth Sci.* **2013**, *3*, B176–B181.
25. Czajkowski, R.; van der Wolf, J.; Krolicka, A.; Ozymko, Z.; Narajczyk, M.; Kaczynska, N.; Lojkowska, E. Salicylic acid can reduce infection symptoms caused by *Dickeya solani* in tissue culture grown potato (*Solanum tuberosum* L.) plants. *Eur. J. Plant Pathol.* **2015**, *141*, 545–558. [[CrossRef](#)]
26. Do, L.T.M.; Aree, T.; Siripong, P.; Pham, T.N.K.; Nguyen, P.K.P.; Tip-Pyang, S. Bougainvinones A-H, peltogynoids from the stem bark of purple *Bougainvillea spectabilis* and their cytotoxic activity. *J. Nat. Prod.* **2016**, *79*, 939–945. [[CrossRef](#)] [[PubMed](#)]
27. Do, L.T.; Aree, T.; Siripong, P.; Vo, N.T.; Nguyen, T.T.; Nguyen, P.K.; Tip-pyang, S. Cytotoxic flavones from the stem bark of *Bougainvillea spectabilis* willd. *Planta Medica* **2018**, *84*, 129–134. [[CrossRef](#)]
28. Jawla, S.; Kumar, Y.; Khan, M.S.Y. Isolation of antidiabetic principle from *Bougainvillea spectabilis* willd (Nyctaginaceae) stem bark. *Trop. J. Pharma. Res.* **2013**, *12*, 761–765. [[CrossRef](#)]
29. Abarca-Vargas, R.; Petricevich, V.L. *Bougainvillea* Genus: A Review on Phytochemistry, Pharmacology, and Toxicology. *Based Complement. Alternat. Med.* **2018**, *2018*, 9070927. [[CrossRef](#)]
30. Narayanan, C.R.; Joshi, D.D.; Mujumdar, A.M.; Dhekne, V.V. Pinitol, a new antidiabetic compound from the leaves of *Bougainvillea spectabilis*. *Curr. Sci.* **1987**, *56*, 139–141.
31. Fawad, S.A.; Khalid, N.; Asghar, W.; Suleria, H.A.R. *In vitro* comparative study of *Bougainvillea spectabilis* “stand” leaves and *Bougainvillea variegata* leaves in terms of phytochemicals and antimicrobial activity. *Chin. J. Nat. Med.* **2012**, *10*, 441–447. [[CrossRef](#)]
32. Khan, M.R.; Siddique, F. Antioxidant effects of *Citharexylum spinosum* in CCl₄ induced nephrotoxicity in rat. *Exp. Toxicol. Pathol.* **2012**, *64*, 349–355. [[CrossRef](#)]
33. Mar, A.; Pripdeevech, P. Chemical composition and antibacterial activity of essential oil and extracts of *Citharexylum spinosum* flowers from Thailand. *Nat. Prod. Commun.* **2014**, *9*, 707–710. [[CrossRef](#)] [[PubMed](#)]
34. Hamed, A.N.E.; Muhammad, M.H.H.; Khalil, H.E.; Kamel, M.S. Biological Studies of *Citharexylum quadrangulare* Jacq. Family Verbenaceae. Assiut Univ. 9th International Pharmaceutical Sciences Conference 2014. Available online: [https://www.researchgate.net/publication/274376180_Assiut_Citharexylum_Bio\(T1\textgreater}\Abstract_form](https://www.researchgate.net/publication/274376180_Assiut_Citharexylum_Bio(T1\textgreater}\Abstract_form) (accessed on 12 March 2014).
35. Mohammed, M.H.H.; Hamed, A.N.E.S.; Khalil, H.E.; Kamel, M.S. Phytochemical and pharmacological studies of *Citharexylum quadrangulare* Jacq. Leaves. *J. Med. Plants Res.* **2016**, *10*, 232–241.
36. Balázs, B.; Toth, G.; Duddeck, H.; Soliman, H.S. Iridoid and lignan glycosides from *Citharexylum spinosum* L. *Nat. Prod. Res.* **2006**, *20*, 201–205.
37. Barizão, E.O.; Visentainer, J.V.; Almeida, V.C.; Ribeiro, D.; Chisté, R.C.; Fernandes, E. *Citharexylum solanaceum* fruit extracts: Profiles of phenolic compounds and carotenoids and their relation with ROS and RNS scavenging capacities. *Food Res. Int.* **2016**, *86*, 24–33. [[CrossRef](#)]

38. Saidi, I.; Waffo-Téguo, P.; Ayeb-Zakhama, A.E.L.; Harzallah-Skhiri, F.; Marchal, A.; Ben Jannet, H. Phytochemical study of the trunk bark of *Citharexylum spinosum* L. growing in Tunisia: Isolation and structure elucidation of iridoid glycosides. *Phytochemistry* **2018**, *146*, 47–55. [CrossRef]
39. Mohamed, A.A.; Behiry, S.I.; Younes, H.A.; Ashmawy, N.A.; Salem, M.Z.M.; Márquez-Molina, O.; Barbabosa-Pilego, A. Antibacterial activity of three essential oils and five monoterpenes against *Ralstonia solanacearum* phylotype II isolated from potato. *Microb. Pathogen.* **2019**, *135*, 103604. [CrossRef]
40. Staley, J.T.; Boone, D.R.; Garrity, G.M.; Devos, P.; Fellow, M.G.; Rainey, F.A.; Schlifer, K.H.; Brenner, D.J.; Castenholz, R.W.; Holt, J.G.; et al. *Bergey's Manual of Systematic Bacteriology*; The Williams and Wilking Company Baltimore Med.: Philadelphia, PA, USA, 2005; Volume 2, p. 469.
41. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiples sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef]
42. Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol. Biol. Evol.* **2011**, *28*, 2731–2739. [CrossRef]
43. Yaganza, E.S.; Arul, J.; Tweddell, R.J. Effect of pre-storage application of different organic and inorganic salts on stored potato quality. *Potato Res.* **2004**, *46*, 167–178. [CrossRef]
44. Sambrook, J.; Fritsch, E.F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory: New York, NY, USA, 1989.
45. Salem, M.Z.M.; Behiry, S.I.; EL-Hefny, M. Inhibition of *Fusarium culmorum*, *Penicillium chrysogenum* and *Rhizoctonia solani* by *n*-hexane extracts of three plant species as a wood-treated oil fungicide. *J. Appl. Microbiol.* **2019**, *126*, 1683–1699. [CrossRef] [PubMed]
46. NIST/EPA/NIH Mass. Spectral Library (NIST 14) and NIST Mass Spectral Search Program (Version 2.0g) May 2014. Available online: <https://www.sisweb.com/software/ms/nist.htm> (accessed on 5 March 2019).
47. Salem, M.Z.M.; Mansour, M.M.A.; Elansary, H.O. Evaluation of the effect of inner and outer bark extracts of Sugar Maple (*Acer saccharum* var. *saccharum*) in combination with citric acid against the growth of three common molds. *J. Wood Chem. Technol.* **2019**, *39*, 136–147. [CrossRef]
48. Abdelsalam, N.R.; Salem, M.Z.M.; Ali, H.M.; Mackled, M.I.; EL-Hefny, M.; Elshikh, M.S.; Hatamleh, A.A. Morphological, biochemical, molecular, and oil toxicity properties of *Taxodium* trees from different locations. *Indu. Crop. Prod.* **2019**, *139*, 111515. [CrossRef]
49. SAS. *Users Guide: Statistics (Release 8.02)*; SAS Institute Inc.: Cary, NC, USA, 2001.
50. Pérombelon, M.C.M.; Kelman, A. Ecology of soft rot Erwinias. *Ann. Rev. Phytopathol.* **1980**, *18*, 361–367. [CrossRef]
51. Zhijian, Z.; Shufen, W.; Qi, G.; Xianping, L.; Yunkun, H. Isolation and identification of bacterial soft rot pathogens from potato tubers in Yunnan province. *J. Yunnan Agric. Univ.* **2000**, *15*, 499–502.
52. Behiry, S.I.; Ashmawy, N.A.; Abdelkhalek, A.A.; Younes, H.A.; Khaled, A.E.; Hafez, E.E. Compatible- and incompatible-type interactions related to defense genes in potato elucidation by *Pectobacterium carotovorum*. *J. Plant Dis. Prot.* **2018**, *125*, 197–204. [CrossRef]
53. EL-Hefny, M.; Ali, H.M.; Ashmawy, N.A.; Salem, M.Z.M. Chemical Composition and Bioactivity of *Salvadora persica* Extracts against Some Potato Bacterial Pathogens. *BioResources* **2017**, *12*, 1835–1849. [CrossRef]
54. Zayed, A.; Maayouf, M. First record of soft rot on imported potato varieties in Great Libyan Jamahiriya. *J. Plant Prot.* **1989**, *7*, 172–173.
55. Choi, J.E.; Man, K.S.; Yu, S.J. Identification of bacteria causing soft rot disease of carrot. *Korean J. Plant Pathol.* **1989**, *5*, 349–353.
56. Chung, K.T.; Stevens, S.E.; Lin, W.F., Jr.; Wie, C.I. Growth inhibition of selected food borne bacteria by tannic acid, propyl gallate and related compounds. *Lett. Appl. Microbiol.* **1993**, *17*, 29–32. [CrossRef]
57. Hodek, P.; Pavel, T.; Marie, S. Flavonoids potent and versatile biologically active compounds interacting with cytochromes p 450. *Chemico-Biol. Interact.* **2002**, *139*, 1–21. [CrossRef]
58. Meddleton, E.; Kandaswami, C.; Theoharides, T.C. The effect of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol. Rev.* **2000**, *52*, 673–751.
59. Lojkowska, E.; Holubovska, M. The role of polyphenol oxidase and peroxidase in potato tuber resistance to soft rot caused by *Erwinia carotovora*. *J. Phytopathol.* **1992**, *136*, 319–328. [CrossRef]

60. Hagerman, A.E. Chemistry of Tannin-Protein Complexation. In *Chemistry and Significance of Condensed Tannins*; Hemingway, R.W., Karchesy, J.J., Branham, S.J., Eds.; Springer: Boston, MA, USA, 1989; pp. 323–333.
61. Liu, I.C.; Hsu, F.L.; Tsai, T.C.; Chan, P.; Liu, J.Y.H.; Thomas, G.N.; Tomlinson, B.; Lo, M.Y.; Lin, J.Y. Antihypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitors of angiotensin converting enzyme. *J. Bacteriol.* **2003**, *73*, 1543–1555.
62. Chung, K.T.; Wong, T.Y.; Wei, C.I.; Huang, Y.W.; Lin, W. Tannins and human health: A review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 421–464. [[CrossRef](#)] [[PubMed](#)]
63. Expert, D. With holding and exchanging iron: Interactions between *Erwinia* spp and their plant hosts. *Annu. Rev. Phytopathol.* **1999**, *37*, 307–334. [[CrossRef](#)] [[PubMed](#)]
64. Hatano, T.; Yoshida, T.; Yoshida, T.; Agata, N.T.; Okuda, T. Effect of interaction of tannins with co-existing substances: Inhibitory effect of tannins and related polyphenols on xanthine oxidase. *Chem. Pharm. Bull.* **1990**, *5*, 1224–1229. [[CrossRef](#)]
65. Leinmüller, E.; Steingass, H.; Henke, K.H. Tannins in ruminant feed stuffs. Ed. Metzinger. *Anim. Res. Dev.* **1991**, *33*, 9–56.
66. Singh, S.; Reddu, S.K.; Sharma, S.K.; Ali, M. New unsaturated fatty acid from roots of *Bougainvillea spectabilis* Willd. *Asian J. Chem.* **2009**, *21*, 4744–4748.
67. Vukovic, N.; Kacaniova, M.; Hleba, L.; Sukdolak, S. Chemical Composition of the Essential oil of *Bougainvillea spectabilis* from Montenegro. *J. Essent. Oil Bear. Plants* **2013**, *16*, 212–215. [[CrossRef](#)]
68. Ali, M.S.; Ibrahim, S.A.; Ahmed, F.; Pervez, M.K. Color versus bioactivity in the flowers of *Bougainvillea spectabilis* (Nyctaginaceae). *Nat. Prod. Res.* **2005**, *19*, 1–5. [[PubMed](#)]
69. Sudipta, K.M.; Lokesh, P.; Rashmi, W.; Vijay, R.; Ssn, K. Phytochemical screening and *in vitro* antimicrobial activity of *Bougainvillea spectabilis* flower extracts. *Inter. J. Phytomed.* **2012**, *4*, 375–379.
70. Chowdhury, F.; Pal, S.; Sharmin, T.; Rashid, R.B.; Sikder, M.A.; Kabir, S.; Rahman, M.S.; Rashid, M.A. Bioactivities of *artocarpus chaplasha roxb.* and *Bougainvillea spectabilis willd.* *Bang. Pharma. J.* **2013**, *16*, 63–68. [[CrossRef](#)]
71. Dhankhar, S.; Sharma, M.; Ruhil, S.; Balhara, M.; Kumar, M.; Chhillar, A.K. Evaluation of antimicrobial and antioxidant activities of *Bougainvillea spectabilis*. *Inter. J. Pharm. Pharma. Sci.* **2013**, *5*, 178–182.
72. Khalifa, T.I.; El-Gendi, O.D.; Ammar, H.A.; El-Naggar, D.M. Iridoid glycosides from *Citharexylum quadrangulare*. *Asian J. Chem.* **2012**, *14*, 197–202.
73. El-Naggar, D.M. Antibilharzial Study of Some Extracts from *Citharexylum quadrangulare* Jacq. Ph.D. Thesis, Al-Azhar University, Cairo, Egypt, 2007.
74. Wei, A.; Shibamoto, T. Antioxidant activities and volatile constituents of various essential oils. *J. Agric. Food Chem.* **2007**, *55*, 1737–1742. [[CrossRef](#)]
75. Mansour, M.M.A.; Salem, M.Z.M.; Khamis, M.H.; Ali, H.M. Natural durability of *Citharexylum spinosum* and *Morus alba* woods against three mold fungi. *BioResources* **2015**, *10*, 5330–5344. [[CrossRef](#)]
76. Zhang, J.H.; Sun, H.L.; Chen, S.Y.; Zeng, L.; Wang, T.T. Anti-fungal activity, mechanism studies on α -Phellandrene and Nonanal against *Penicillium cyclopium*. *Bot. Stud.* **2017**, *58*, 13. [[CrossRef](#)]
77. Fernando, W.G.D.; Ramarathnam, R.; Krishnamoorthy, A.S.; Savchuk, S.C. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. *Soil Biol. Biochem.* **2005**, *37*, 955–964. [[CrossRef](#)]
78. Rodriguez-Burbano, D.; Quijano-Celis, C.; Pino, J. Composition of the essential oil from leaves of *Astronium graveolens* Jacq grown in Colombia. *J. Essent. Oil Res.* **2010**, *22*, 488–489. [[CrossRef](#)]
79. Pandey, A.K.; Mohan, M.; Singh, P.; Palni, U.T.; Tripathi, N.N. Chemical composition, antibacterial and antioxidant activity of essential oil of *Eupatorium adenophorum* Spreng. from Eastern Uttar Pradesh, India. *Food Biosci.* **2014**, *7*, 80–87. [[CrossRef](#)]
80. Ashmawy, N.A.; Salem, M.Z.M.; EL-Hefny, M.; Abd El-Kareem, M.S.M.; El-Shanhorey, N.A.; Mohamed, A.A.; Salem, A.Z.M. Antibacterial activity of the bioactive molecules identified in three woody plants against some pathogenic bacteria. *Microb. Pathogen.* **2018**, *121*, 331–340. [[CrossRef](#)]
81. Tenfen, A.; Cechinel-Zanchett, C.C.; Dalmagro, A.P.; Zimath, P.; Boeder, A.M.; Santos, G.M.D.; Campos, A.; Sibert, D.A.; Micke, G.; Vitali, L.; et al. Biological potential of *Citharexylum myrianthum* Cham. leaves *in vitro* and phenolic profile by HPLC-ESI-MS/MS. *J. Appl. Pharma. Sci.* **2018**, *8*, 74–80.
82. Ashmawy, N.A.; Al Farraj, D.A.; Salem, M.Z.M.; Elshikh, M.S.; Al-Kufaidy, R.; Alshammari, M.k.; Salem, A.Z.M. Potential impacts of *Pinus halepensis* Miller trees as a source of phytochemical compounds: Antibacterial activity of the cones essential oil and n-butanol extract. *Agrofores. Syst.* **2018**. [[CrossRef](#)]

83. Droby, S.; Eick, A.; Macarasin, D.; Cohen, L.; Rafaela, G.; Stange, R.; McColum, G.; Dudai, N.; Nasser, A.; Wisniewski, M.; et al. Role of citrus volatiles in host recognition, germination and growth of *Penicillium digitatum* and *Penicillium italicum*. *Postharvest Biol. Technol.* **2008**, *49*, 386–396. [[CrossRef](#)]
84. Inouye, S.; Takizawa, T.; Yamaguchi, H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *J. Antimicrob. Chemother.* **2001**, *47*, 565–573. [[CrossRef](#)] [[PubMed](#)]
85. Farag, R.S.; Daw, Z.Y.; Hewedi, F.M.; El-Baroty, G.S.A. Antimicrobial activity of some Egyptian spice essential oils. *J. Food Protect.* **1989**, *52*, 665–667. [[CrossRef](#)] [[PubMed](#)]
86. Smith-Palmer, A.; Stewart, J.; Fyfe, L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett. Appl. Microbiol.* **1998**, *26*, 118–122. [[CrossRef](#)]
87. Kubo, A.; Lunde, C.S.; Kubo, I. Antimicrobial activity of the olive oil flavor compounds. *J. Agric. Food Chem.* **1995**, *43*, 1629–1633. [[CrossRef](#)]
88. Bisignano, G.; Lagana', M.G.; Trombetta, D.; Arena, S.; Nostro, A.; Uccella, N.; Mazzanti, G.; Saija, A. *In vitro* bacterial activity of some aliphatic aldehydes from *Olea europaea* L. *FEMS Microbiol. Lett.* **2001**, *198*, 9–13. [[CrossRef](#)]
89. Mohamed, A.A.; Behiry, S.I.; Ali, H.M.; EL-Hefny, M.; Salem, M.Z.M.; Ashmawy, N.A. Phytochemical Compounds of Branches from *P. halepensis* Oily Liquid Extract and *S. terebinthifolius* Essential Oil and Their Potential Antifungal Activity. *Processes* **2020**, *8*, 330. [[CrossRef](#)]
90. Tassou, C.C.; Nychas, G.J.E. Antimicrobial activity of the essential oil of Mastic gum (*Pistacia lentiscus* var *chia*) on gram-positive and gram-negative bacteria in broth and model food systems. *Int. Biodeterior. Biodegr.* **1995**, *36*, 411–420. [[CrossRef](#)]
91. Mann, C.M.; Cox, S.D.; Markham, J.L. The outer membrane of *Pseudomonas aeruginosa* NCTC6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Lett. Appl. Microbiol.* **2000**, *30*, 294–297. [[CrossRef](#)] [[PubMed](#)]
92. Bajpai, V.K.; Sharma, A.; Baek, K.H. Antibacterial mode of action of *Cudrania tricuspidata* fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. *Food Control.* **2013**, *32*, 582–590. [[CrossRef](#)]
93. Tsuchiya, H. Biphasic effects of acetaldehyde-biogenic amine condensation products on membrane fluidity. *J. Pharm. Pharmacol.* **2001**, *53*, 121–127. [[CrossRef](#)]
94. Marquis, R.E.; Clock, S.A.; Mota-Meira, M. Fluoride and organic weak acids as modulators of microbial physiology. *FEMS Microbiol. Rev.* **2003**, *26*, 493–510. [[CrossRef](#)]
95. Helal, G.A.; Sarhan, M.M.; Abu Shahla, A.N.K.; Abou El-Khair, E.K. Effects of *Cymbopogon citratus* L. essential oil on the growth, lipid content and morphogenesis of *Aspergillus niger* ML2-strain. *J. Basic. Microb.* **2006**, *46*, 456–469. [[CrossRef](#)]
96. Helal, G.A.; Sarhan, M.M.; Abu, S.A.N.K.; Abou, E.E.K. Effects of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *Aspergillus flavus* ML2-strain. *J. Basic Microb.* **2007**, *47*, 5–15. [[CrossRef](#)]
97. Dias, M.I.; Sousa, M.J.; Alves, R.C.; Ferreira, I.C.F.R. Exploring plant tissue culture to improve the production of phenolic compounds: A review. *Ind. Crops Prod.* **2016**, *82*, 9–22. [[CrossRef](#)]
98. Pereira, D.M.; Valentão, P.; Pereira, J.A.; Andrade, P.B. Phenolics: From chemistry to biology. *Molecules* **2009**, *14*, 2202–2211. [[CrossRef](#)]
99. Pragasam, S.J.; Murunikara, V.; Sabina, E.P.; Rasool, M. Ameliorative effect of p-coumaric acid, a common dietary phenol, on adjuvant-induced arthritis in rats. *Rheumatol. Int.* **2013**, *33*, 325–334. [[CrossRef](#)] [[PubMed](#)]
100. Heleno, S.A.; Martins, A.; Queiroz, M.J.R.P.; Ferreira, I.C.F.R. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chem.* **2015**, *173*, 501–513. [[CrossRef](#)] [[PubMed](#)]
101. Pei, K.; Ou, J.; Huang, J.; Ou, S. p-Coumaric acid and its conjugates: Dietary sources, pharmacokinetic properties and biological activities. *J. Sci. Food Agric.* **2016**, *96*, 2952–2962. [[CrossRef](#)] [[PubMed](#)]
102. Yang, W.S.; Jeong, D.; Yi, Y.; Park, J.G.; Seo, H.; Moh, S.H.; Hong, S.; Cho, J.Y. IRAK1/4-targeted anti-inflammatory action of caffeic Acid. *Mediat. Inflamm.* **2013**, *2013*, 518183. [[CrossRef](#)] [[PubMed](#)]
103. Teodoro, G.R.; Ellepola, K.; Seneviratne, C.J.; Koga-Ito, C.Y. Potential use of phenolic acids as anti-candida agents: A review. *Front. Microbiol.* **2015**, *6*, 1–11. [[CrossRef](#)] [[PubMed](#)]
104. Joshi, J.R.; Burdman, S.; Lipsky, A.; Yedidia, I. Effects of plant antimicrobial phenolic compounds on virulence of the genus *Pectobacterium*. *Res. Microbiol.* **2015**, *166*, 535–545. [[CrossRef](#)]

105. Martins, N.; Barros, L.; Henriques, M.; Silva, S.; Ferreira, I.C.F.R. Activity of phenolic compounds from plant origin against *Candida* species. *Ind. Crops Prod.* **2015**, *74*, 648–670. [[CrossRef](#)]
106. Parkar, S.G.; Stevenson, D.E.; Skinner, M.A. The potential influence of fruit polyphenols on colonic microflora and human gut health. *Int. J. Food Microbiol.* **2008**, *124*, 295–298. [[CrossRef](#)]
107. Karunanidhi, A.; Thomas, R.; Van Belkum, A.; Neela, V. *In vitro* antibacterial and antibiofilm activities of chlorogenic acid against clinical isolates of *Stenotrophomonas maltophilia* including the Trimethoprim/Sulfamethoxazole resistant strain. *BioMed Res. Int.* **2013**, *2013*, 392058. [[CrossRef](#)]
108. Jeong, J.M.; Lee, K.I.; Kim, S.M. Simultaneous determination of benzoic Acid, caffeic acid and chlorogenic acid in seeds of *Eriobotrya japonica* and their antibacterial effect. *J. Appl. Biol. Chem.* **2014**, *57*, 89–93.
109. Wang, J.; Lou, J.; Luo, C.; Zhou, L.; Wang, M.; Wang, L. Phenolic compounds from *Halimodendron halodendron* (Pall.) voss and their antimicrobial and antioxidant activities. *Int. J. Mol. Sci.* **2012**, *13*, 11349–11364. [[CrossRef](#)] [[PubMed](#)]



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