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Bioactivity of Selected Phenolic Acids and Hexane Extracts from *Bougainvilla 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 ata 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*; *Bougainvilla*; *Citharexylum* extract; ecofriendly environmental agents; phenolic acids

1. Introduction

Potato (*Solanum tubersum* 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, H_2S 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 Throught 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′-CTACGGCTACCTTGT GTTACGA-3′). PCR amplification was performed in a total volume of 50 µL, containing 25 µL of master mix (enzymocis, korea), 2 µL of each P0 or P6 primer (10 pmol) with final concentration 0.1–0.5 µM of each primer, 2 µL (50 ng/µL) of bacterial genomic DNA, and molecular grade water was added until the volume reached 50 µL. 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 powered 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 µg/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 µg/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 × 0.25 mm × 0.25 µm 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 ± SD
Pectobacterium	PCC1	Tuber	Roseta	El-Nubaria, El-Behira, Egypt	86.04 ± 0.97
Dickeya	Ds1	Stem	Hermes	Wadi elnatron, El-Behira, Egypt	71.62 ± 0.53
Control					0.00 ± 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	
	Pectobacterium carotovorum* subsp. *carotovorum	***Dickeya solani***
Shape (rods)	+	+
Gram staining	−	−
Motility	+	+
Anaerobic growth	+	+
Potato soft rot	+	+
Growth at 37 °C	+	+
Gelatin liquefaction	+	+
Mucoid growth	+	+
Kovac's oxidase	−	−
H2S 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
p-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 µg/mL	Inhibition Zone Diameter (mm) ± SE	
		PCC1	Ds1
Chlorogenic acid	25	0.00	0.00
	50	0.00	0.00
	100	0.00	0.00
	200	6 ± 0.00	11.5 ± 0.00
	400	10 ± 0.00	15.5 ± 0.00
	800	13.5 ± 0.28	22 ± 0.28
	1600	18.5 ± 0.28	25.5 ± 0.00
	3200	19.25 ± 1.01	25.25 ± 0.43
Ferulic acid	25	0.00	6 ± 0.00
	50	0.00	9 ± 0.00
	100	6.5 ± 0.28	11.5 ± 0.28
	200	7 ± 0.00	14.75 ± 0.14
	400	8.5 ± 0.00	18.5 ± 0.00
	800	12.25 ± 0.14	22.5 ± 0.00
	1600	17.75 ± 0.14	24.25 ± 0.14
	3200	21.5 ± 0.57	25.75 ± 0.14
Bougainvillea spectabilis bark	125	6.66 ± 0.88	6.83 ± 0.16
	250	7.33 ± 0.66	7.16 ± 0.16
	500	7.33 ± 0.33	9.33 ± 0.46
	1000	9 ± 0.57	10 ± 0.33
	2000	9.66 ± 0.33	11 ± 0.22
	4000	12 ± 0.57	12.33 ± 0.33
Citharexylum spinosum wood	125	6.33 ± 0.88	6.16 ± 0.44
	250	6.66 ± 0.66	6.5 ± 0.28
	500	6.66 ± 0.66	7.5 ± 0.28
	1000	7 ± 0.57	7.83 ± 0.16
	2000	8.66 ± 0.33	8.33 ± 0.33
	4000	10 ± 0.57	8.5 ± 0.28
Control	0	0.00	0.00
p-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 Ds1was increased. The highest IZ (12 mm) against PCC1 was observed for *B. spectabilis* bark HeOE applied at a concentration of 4000 µg/mL, followed by the same HeOE with an IZ of 9.66 mm at a concentration of 2000 µg/mL. Furthermore, *B. spectabilis* bark HeOE at 4000, 2000, and 1000 µg/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 µg/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-al (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-al	2.68	770	823
trans-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
cis-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 byGC/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
trans-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, linaool, 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 pulchelloside, 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. cyclopium* [76].

The inhibitory effect of *B. spectabilis* extract could correlate with the concentration of nonanal *versa C. spinosum* wood extracts, and these results are the same as other reports [77–79]. Inhibition against *A. niger* and *P. selerotigenum* 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]. ρ-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-nonenalhave 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, (2*E*)-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 caffeine, 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 carorovorum* 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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