Microbial Pathogenesis 113 (2017) 348–356

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Antibacterial activities of the phytochemicals-characterized extracts of *Callistemon viminalis*, *Eucalyptus camaldulensis* and *Conyza dioscoridis* against the growth of some phytopathogenic bacteria

Mervat EL-Hefny[a], Nader A. Ashmawy[b], Mohamed Z.M. Salem[c], Abdelfattah Z.M. Salem[d,*]

[a] Department of Floriculture, Ornamental Horticulture and Garden Design, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt
[b] Department of Plant Pathology, Faculty of Agriculture (ELShatby), Alexandria University, Alexandria, Egypt
[c] Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt
[d] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Estado de México, Mexico

ARTICLE INFO

Keywords:
Callistemon viminalis
Conyza dioscoridis
Eucalyptus camaldulensis
Phytopathogenic bacteria
Phytochemicals

ABSTRACT

Three bacterial isolates were isolated from infected potato tubers showing soft and brown rots like symptoms as well as one isolate from infected peach tree showing crown gall symptom. The morphological, biochemical and molecular assays proved that bacterial isolates belonging to *Pectobacterium carotovorum* subsp. *carotovorum*, *Ralstonia solanacearum*, *Dickeya* spp. and *Agrobacterium tumefaciens*. The acetone (AcE) and *n*-butanol (ButE) extracts of *Callistemon viminalis* flowers and essential oil from aerial parts of *Conyza dioscoridis* as well as ButE of *Eucalyptus camaldulensis* bark are evaluated at different concentrations against the growth of the isolated bacteria. The diameter of inhibition zone (IZ) and the minimum inhibitory concentrations (MICs) are compared. Results indicated that the highest IZ values were 20.0 mm and 18.3 mm for *E. camaldulensis* bark ButE and *C. viminalis* flower ButE, respectively, against *P. carotovorum*; 16.3 mm and 16.0 mm for *E. camaldulensis* bark ButE and *C. viminalis* flower ButE, respectively, against *R. solanacearum*; 18.5 mm for *C. viminalis* flower AcE and *C. dioscoridis* aerial parts EO against *Dickeya* spp.; and 15.0 mm for *C. viminalis* flower AcE against *A. tumefaciens*. MICs ranged from < 16 μg/mL for *D. solani* to > 4000 μg/mL for *A. tumefaciens*. It was proved that *C. viminalis* flowers AcE contains mainly 5-hydroxymethylfurfural (20.6%), palmitic acid (18.5%), and pyrogallol (16.4%); while *C. viminalis* flower ButE contains palmitic acid (36.3%), 2-hydroxymyristic acid (9.4%), 5-hydroxymethylfurfural (7.2%), and shikimic acid (6.6%); whereas *E. camaldulensis* bark ButE contains 8-nonynoic acid methyl ester (45.6), camphor (30.9%), menthol (8.8%), and 1,8-cineole (eucalyptol) (8.2%), whilst the EO of *C. dioscoridis* aerial parts comprises *Z*-(13,14-epoxy)tetradec-11-en-1-ol acetate (11.6%), γ-elemene (10.2%), tau.-muurolol (7.1%), and cadina-3,9-diene (4.7%). It can be concluded that phytochemical extracts of *C. viminalis*, *E. camaldulensis* and *C. dioscoridis* demonstrated strong to moderate antibacterial effects against the studied plant bacterial pathogens.

1. Introduction

Bacterial pathogens causes many problems to plants and fruits such as brown rot in potato [1], bacterial wilt in tomato [2] caused by *Ralostonia solanacerum*, and soft rot and blackleg caused by *Pectobacterium*, *Dickeya*, *Enterobacter*, and *Bacillus* species [3–5]. Additionally, diseases such as blackleg, rotting of potato stems in the field as well as soft rot in seed tubers during storage were also reported [6,7]. Crown gall is a wide spreed and desteructive plant disease that may significantly reduce vigour and yield of many crops. The aromatic plants have been reported as rich sources of secondary chemical products and their derivatives, hence, they are used as natural biocides against certain pathogens [5,8–13].

Recently, extracts and essential oils from different parts of *Callistemon viminalis* (Sol. ex. Gaertn), G. Don. (*Melaleuca viminalis* (Sol. ex Gaertn.) Byrnes) have been studied [14–16]. The aqueous extract of flowers and leaves exhibited an antibacterial activity against the Gram-positive bacteria [14] while, water and ethanol extracts of inflorescence of *C. viminalis* demonstrated strong anti-quorum sensing activities against *Chromobacterium violaceum* and *A. tumefaciens* [17]. The major constituent of *C. viminalis* essential oil is 1,8-cineole [10,16], and the polar extracts contain alkaloids, flavonoids and some phenols whereas, the non-polar extracts contain tannins, terpenes and quinines [18]. Several extracts from different parts of *Eucalyptus camaldulensis* L. are

* Corresponding author.
E-mail address: asalem70@yahoo.com (A.Z.M. Salem).

https://doi.org/10.1016/j.micpath.2017.11.004
Received 15 October 2017; Received in revised form 31 October 2017; Accepted 2 November 2017
Available online 07 November 2017
0882-4010/

distinguished for their biological activities and characterized by the presence of bioactive compounds such as tannins, flavonoids, essential oils, terpenoids, and phenolics [19,20].

Conyza dioscoridis L. Desf. or *Pluchea dioscoridis* L. DC. (Asteraceae family) is commonly used in folk medicine as rheumatic pains relief, carminative and colic. It can also be used as, anti-inflammatory, anti-oxidant, antihyperglycemic, antiulerogenic, antimicrobial, anti-diarrheal, antinociceptive and antipyretic drug [21,22]. Useful components such as flavonoids, steroids, essential oil and sesquiterpenoids were isolated from *C. dioscoridis* [21,23]. Other chemical compounds like 15-hydroxyisocostic acid, methyl 15-oxo-eudesome-4, 11(13)-diene 12-oate and 1α, 9α-dihydroxy-α-cyclocostunolide were also isolated from the leaves [24].

The present study aims to evaluate the antibacterial effects of the extracts of *C. viminalis* flowers, aerial parts of *C. dioscoridis* and *E. camaldulensis* bark grown in Egypt against the growth of some phyto-pathogenic bacteria. Furthermore, the chemical compositions of the studied extracts are investigated using Gas chromatography–mass spectrometry (GC/MS).

2. Materials and methods

2.1. Plant material

Flowers of *Callistemon viminalis*, aerial parts of *Conyza dioscoridis* and *Eucalyptus camaldulensis* bark were collected during August 2016 from different location at Alexandria City, Egypt and obtained voucher numbers at the Egyptian barcode of the Life Project (www.egyptbol.org), Faculty of Agriculture, Alexandria University. All the materials were air-dried under laboratory conditions for two weeks and then ground into 0.2–0.4 mm powder.

2.2. Preparation of extracts and essential oil

Fifty g of the dried powder materials of *C. viminalis* flowers and *E. camaldulensis* bark were extracted by soaking with 150 mL of *n*-butanol (But). Another 50 g of dried *C. viminalis* flowers were extracted by acetone (Ac) at laboratory temperature under shaking for 6 h according to Salem et al. [10] with some modifications. For the extraction of essential oil (EO) from the air-dried aerial parts of *C. dioscoridis*, 100 g were extracted by hydro-distillation method for 3 h [25] using a Clevenger extractor [13]. The oil was dried over anhydrous Na_2SO_4, then measured (0.3 mL/100 g dried aerial parts). The acetone (AcE) and *n*-butanol (ButE) extracts were allowed to evaporate under reduced pressure at 45 °C with a rotary evaporator, and stored at 4 °C until further use. The yields of ButE from *C. viminalis* flowers and *E. camaldulensis* bark, and the AcE of *C. viminalis* flowers were 7.15, 12.8, and 9.30 g/100 air-dried samples, respectively.

2.3. Isolation of bacterial pathogens

2.3.1. Isolation of soft rot pathogen

Naturally infected potato tuber and stem samples showing typical symptoms of soft rot or blackleg disease were collected from fields and stores from different locations of Egypt (Fig. 1). Diseased tubers were first washed with tap water then surface sterilized with 1% sodium hypochlorite solution (NaOCl) for 3 min then washed thoroughly 3 times with sterilized distilled water; the rotted tissues of tuber were put into sterilized mortar and homogenized then left to stand for 20 min then a loopful of the resulting suspension was streaked into plates containing Glycerol nutrient agar (GNA) medium according to Abo-El-Dahab and El-Goorani [26].

2.3.2. Isolation of brown rot pathogen

Isolation trails were conducted from infected potato tubers showing internal symptoms of brown rot disease (Fig. 2). Infected tubers were segmented into small pieces and placed in test tubes containing 5 mL of sterile distilled water for standard isolation [27]. Bacteria were allowed to flow from the vascular bundles for 5–10 min. One loopful of the bacterial suspension was streaked onto 2, 3, 5 triphenyl tetrazolium chloride (TZC) agar medium consisted of 10 g of peptone, 1 g of casamino acid (casein hydrolysate), 5 g of glucose and 18 g of agar in 1 L of distilled water and incubated at 28 °C for 48 h [28].

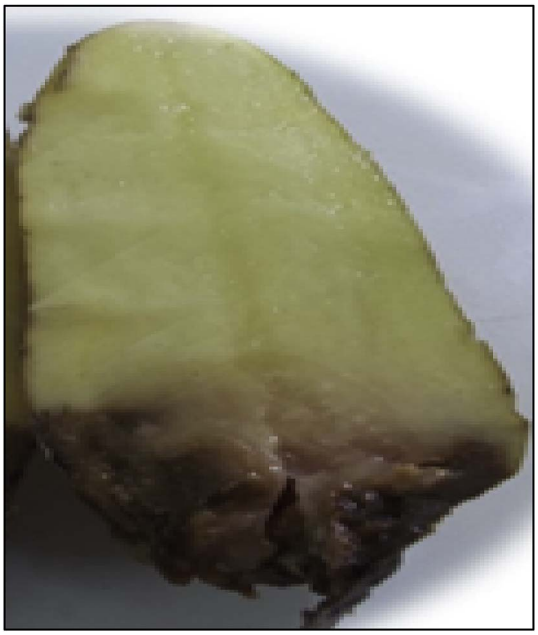

Fig. 1. Natural infection of potato by soft rot bacteria (*Pectobacterium* and *Dickeya* spp.).

Fig. 2. *Ralstonia solanacearum* bacterial oozes in naturally infected potato tuber.

2.3.3. Isolation of crown gall pathogen

Isolation trial was carried out from tumours formed on Peach (*Prunus persica*) trees (Fig. 3). Soft galls were washed under tap water, surface sterilized in 10% sodium hypochlorite solution for 10 min. A small portion of soft galled tissues were cut under aseptic conditions with sterile scalpel and forceps to crush in mortar with a few drops of distilled water to make a suspension of the bacteria. A loopful of the resulting suspension was streaked over the surface of glycerol nutrient

Fig. 3. Crown gall caused by *Agrobacterium tumefaciens* in naturally infected Peach.

agar (GNA) medium (5 g Peptone, 3 g beef extract, 20 mL glycerol and 20 g agar in distilled water up to1L) [29] in Petri dishes, colonies were observed after 48 h incubation at 27 °C. Colonies were isolated and purified through the single colony technique.

2.4. Identification of bacterial isolates

2.4.1. Morphological, physiological and biochemical tests

Single colonies of each isolate of total 4 isolates were identified by standard, morphological, physiological and biochemical methods for soft rot and blackleg bacteria [30], brown rot bacterium [31,32], and crown gall pathogen [33].

2.4.2. Molecular identification of bacterial pathogens

2.4.2.1. DNA extraction protocol. Bacterial isolates were grown overnight in LB medium at 28 °C with constant shaking at 200 rpm. Cells from 3 mL culture were pelleted by centrifugation at 6000*g* for 5 min using a microcentrifuge and DNA was isolated according to Ausubel et al. [34].

2.4.2.2. A species-specific polymerase chain reaction (PCR) assay. PCR-based methods were used for specific identification of the four bacterial isolates, these methods are based on specific amplification of a target DNA sequence that is unique to a bacterial genome described in (Table 1). The reaction was determined to be 50 ng/μL using spectrophotometer (MaestroNano Drop MN-913) for pure genomic DNA per 25 μl reaction volume consisting of 12.5 μL of 1x PCR Green Master Mix (Thermo Scientific™), 3 μL of genomic bacterial DNA, 0.5 μL of each primer (10 μM) [35]. The amplification was performed using thermocycler (Techne, UK) with annealing temperature to each primer as listed (Table 1). The PCR product were separated by electrophoresis in 1.5% agarose gel containing ethidium bromide solution and visualized under UV-transilluminator.

2.5. Antibacterial activity

The antibacterial activity of different extracts and essential oil was evaluated against the growth of isolated soft rot pathogens *P. carotovorum* subsp. *carotovorum*, and *Dicheya* spp. and brown rot pathogen *R. solanacearum*, and crown gall *A. tumefaciens* using the Kirby-Bauer disc diffusion susceptibility test [40]. GNA medium was used as a culture medium for maintenance of the bacterial test organisms [26]. Solid GNA medium was used for screening the antibacterial activities. The GNA plates were prepared by pouring 15 mL of melted media into sterile Petri dishesand allowed to solidify for 5 min. Using sterile cotton swabs, 0.5 mL of fresh 24- hour's old bacterial suspension (ca. 10^8 CFU/mL) was spread over the surface of GNA plates.

Sterile plain discs of 4 mm diameter (Whatman filter paper no. 1) were placed on the surface of agar plates and each disc was loaded with 20 μL of either the concentrated extract or the essential oil (4000, 2000, 1000, 500, 250 and 125 μg/mL) dissolved in 10% of dimethyl sulfoxide. The plates were incubated at 30 °C for 24 h. The diameters of the inhibition zones (IZs) were measured in millimeters. All tests were performed in three replicates. Negative control discs were prepared using 10% of dimethyl sulfoxide. Gentamicin (20 μg/disc) was used as a positive control. Minimum inhibitory concentrations (MICs) using serial dilutions of the extracts and essential oil ranged between 16 and 4000 μg/mL were performed in 96-well micro-plates [41].

2.6. GC/MS analyses of extracts and essential oil

The chemical compositions of acetone and *n*-butanol extracts of *C. viminalis* flowers and *E. camaldulensis* bark were analyzed using a Trace gas chromatography (GC) Ultra-ISQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 μm film thickness) apparatus. The column oven temperature was initially adjusted to 120 °C and then increased by 5 °C/min up to 200 °C, which was held for 2 min, then increased by 10 °C/min up to 280 °C. The injector and detector (mass spectra transfer line) temperatures were kept at 250 °C. Helium, the carrier gas, was kept in a constant flow rate of 1 mL/min. The solvent delay time was 2 min. Diluted samples of 1 μL were injected automatically using an Auto-sampler AS3000 coupled with the GC unit in the split mode. Electron impact ionization (EI mass spectra) was collected at 70 electron volt over the *m/z* range from 40 to 550 in full scan mode.

Table 1
Primers, thermocycling conditions and expected amplicon length for identification of tested bacterial species.

Primers	Nucleotide Sequence 5′–3′	Annealing (°C)	Amplicon Length (bp)	Target Bacteria	References
EXpccF	GAACTTCGCACCGCCGACCTTCTA	60	550	*Pectobacterium cartovorum* subsp. *cartovorum*	[36]
EXpccR	GCCGTAATTGCCTACCTGCTTAAG				
5A	GCGGTTGTTCACCAGGTGTTTT	60	500	*Dickeya* spp.	[37]
5B	ATGCACGCTACCTGGAAGTAT				
OLI-1	GGGGG TAGCTTGCTACCTGCC	68	288	*Ralstonia solanacearum*	[38]
Y2	CCCACTGCTGCCTCCCGTAG GAGT				
tms2A	CGCCACACAGGGCTGGGGGTAGGC	67	220	*Agrobacterium tumefaciens*	[39]
tms2B	GGAGCAGTGCCGGGTGCCTCGGGA				

The EO from *C. dioscoridis* aerial parts was analyzed using a Trace GC Ultra/Mass spectrophotometer ISQ (Thermo Scientific) instrument equipped with a flame ionization detector and a DB-5 narrow bore column (length 10 m × 0.1 mm ID, 0.17 μm film thickness; Agilent, Palo Alto, CA, USA) was used. Helium was used as the carrier gas (flow rate of 1 mL/min), and the oven temperature elevated from 45 to 165 °C (4 °C/min) and from 165 to 280 °C (15 °C/min) with post run (off) at 280 °C. Samples (1 μL) were injected at 250 °C, with split/splitless injector (50:1 split ratio) in the splitless mode flow with 10 mL/min as described in Salem et al. [25]. Identification of the constituents was performed on the basis of mass spectra library search (NIST and Wiley) [42].

2.7. Statistical analysis

Values of the inhibition zone (mm as mean ± standard diviation) were analyzed using factorial experiment in ANOVA test with two factors; the extracts and the extract concentrations using the GLM procedure [43].

3. Results

3.1. Isolation and identification of bacterial isolates

Three bacterial isolates were isolated from infected potato tubers and one isolate from infected peach tree. Morphological, physiological and biochemical characteristics of the four tested bacterial isolates indicated that isolates were belonging to *Pectobacterium carotovrum*, *Dickeya* spp., *Ralstonia solanacearum* and *Agrobacterium tumefaciens*. The four bacterial isolates were confirmed and identified at the molecular level based on the PCR analyses using four pairs of specific primers (Table 1) that produced a 550 bp specific PCR product for *P. carotovrum* subsp. *carotovorum*, 500 bp for *Dickeya* spp., 288 bp amplicon for *R. solanacearum* and 220 bp for *A. tumefaciens*.

3.2. Antibacterial activity of extracts

The effects of different extracts and their concentrations, as well as the interaction between them were highly significant on the diameter of inhibition zones (IZs) against the growth of *P. carotovorum*, *R. solanacearum*, *Dickeya* spp. and *A. tumefaciens*, as shown in Figs. 4 and 5.

In Table 2, the highest IZs were reported against the growth of *P. carotovorum* by applying 4000 μg/mL of the extracts. IZ values were 20.0 mm, 18.3 mm, and 15.5 mm for *E. camaldulensis* bark ButE, *C. viminalis* flower ButE, and *C. dioscoridis* aerial parts EO respectively. Whereas, ButEs from *E. camaldulensis* bark and *C. viminalis* flower showed the highest IZ values with 16.3 mm and 16.0 mm respectively, followed by the AcE from *C. viminalis* flower and *C. dioscoridis* aerial parts EO against the growth of *R. solanacearum* at the above concentration.

4000 μg/mL of AcE from *C. viminalis* flower and *C. dioscoridis* aerial parts EO gave IZ value of 18.5 mm thus, showing the highest activity against the growth of *Dickeya* spp.

The AcE and ButE from *C. viminalis* flowers showed the highest IZ values against the growth of *A. tumefaciens*, when applying the extracts at the concertation of 4000 μg/mL.

According to the MIC values reported in Table 3, the highest activities were 16 μg/mL for *E. camaldulensis* bark ButE against the growth of *P. carotovorum*, 64 μg/mL for *C. viminalis* flower AcE against *R. solanacearum*, with, < 16 μg/mL for *C. viminalis* flower AcE and *C. dioscoridis* aerial parts EO against *D. solani* and 16 μg/mL *for C. viminalis* flower AcE with *A. tumefaciens*.

3.3. Phytochemical constituents of extracts and essential oil

Table 4 presents the phytochemical constituents of *C. viminalis* flowers AcE where, the main compounds are 5-hydroxymethylfurfural (20.6%), palmitic acid (18.5%), pyrogallol (16.3%), *β*-terpinyl acetate (5.1%), 4-heptenal (4.6%), 5-methyl furfural (4.3%), bicyclo[2.2.1] heptane-1-methanol,7,7-ethylenedioxy (4.3%), γ-heptalactone (3.6%), and aspidinol (3.1%).

The phytochemical constituents of *C. viminalis* flower ButE are presented in Table 5. The main abundant compounds are palmitic acid (36.4%), 2-hydroxymyristic acid (9.4%), 5-hydroxymethylfurfural (7.2%), shikimic acid (6.6%), 4-*O*-*α*-D-galactopyranosyl-*α*-D-glucopyranose (5.8%), desulphosinigrin (5.6%), 1,1-dibutoxybutane (4.2%), 1-butoxy-1-methoxybutane (2.5%), 2-methyl-4-isovalerylphloroglucinol (2.3%), and (*Z*)-2-pentenal (2.3%).

Table 6 shows the chemical constitutes of *E. camaldulensis* bark ButE where, The main compounds are 8-nonynoic acid methyl ester (45.6), camphor (30.9%), menthol (8.8%), 1,8-cineole (eucalyptol) (8.2%), and isobornyl acetate (2.6%). α-pinene and limonene are present in

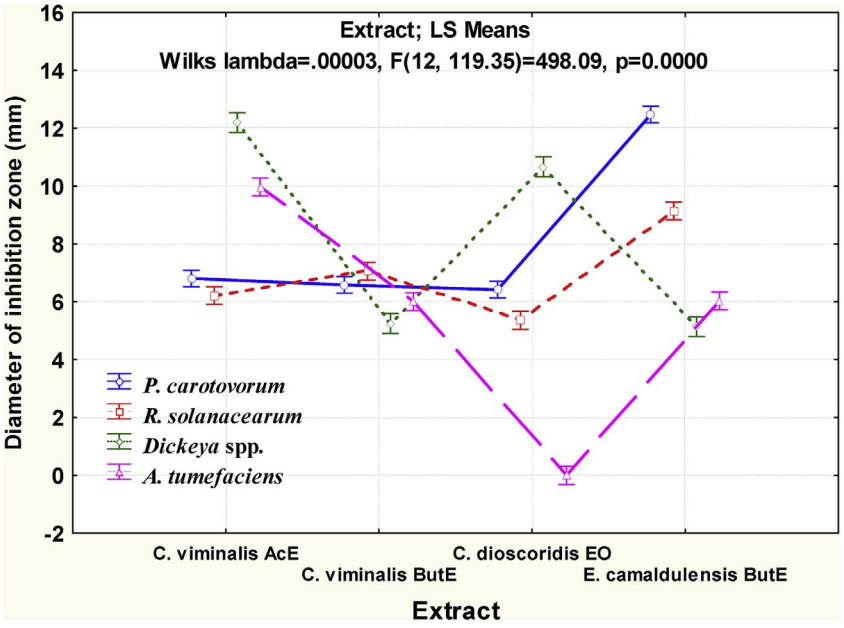

Fig. 4. Effect of extracts type on the diameter of inhibition zone of bacterial growth.

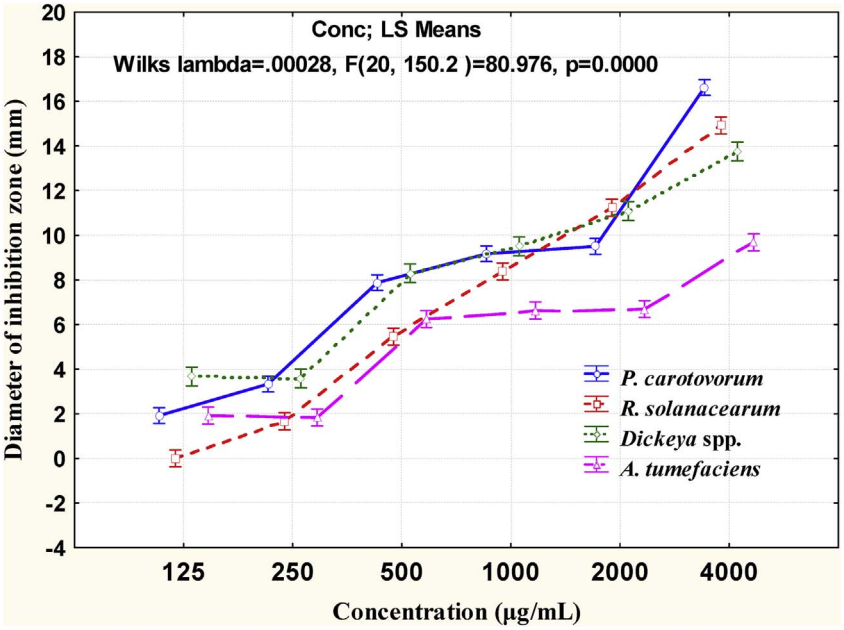

Fig. 5. Effect of different concentrations of extracts on the diameter of inhibition zone of bacterial growth.

Table 2
Values of antibacterial activity of extracts from *C. viminalis*, *C. dioscoridis* and *E. camaldulensis*.

Extract	Con. (µg/mL)	Diameter of inhibition zones[a] (mm ± standard division)			
		P. carotovorum	*R. solanacearum*	*Dickeya* spp.	*A. tumefaciens*
C. viminalis flower AcE	4000	12.67 ± 0.58	13.33 ± 1.53	18.50 ± 0.50	15.07 ± 0.50
	2000	7.33 ± 0.58	10.77 ± 0.68	15.50 ± 0.50	11.12 ± 0.93
	1000	7.67 ± 0.29	7.33 ± 0.58	12.00 ± 1.00	10.33 ± 0.76
	500	7.50 ± 0.50	5.83 ± 0.76	10.83 ± 0.76	8.33 ± 0.76
	250	5.67 ± 0.58	0.00	8.00 ± 1.00	7.33 ± 0.58
	125	0.00	0.00	8.33 ± 0.58	7.67 ± 1.15
C. viminalis flower ButE	4000	18.33 ± 0.58	16.00 ± 1.00	10.00 ± 0.87	13.33 ± 0.58
	2000	7.67 ± 0.58	11.00 ± 1.00	7.17 ± 0.76	7.33 ± 0.58
	1000	7.17 ± 0.76	8.00 ± 1.00	7.67 ± 0.58	7.67 ± 1.15
	500	6.33 ± 0.58	7.33 ± 0.58	6.67 ± 0.58	7.67 ± 1.15
	250	0.00	0.00	0.00	0.00
	125	0.00	0.00	0.00	0.00
C. dioscoridis aerial parts EO	4000	15.50 ± 0.50	14.00 ± 1.00	18.50 ± 0.50	0.00
	2000	8.00 ± 1.00	10.50 ± 0.50	13.50 ± 0.50	0.00
	1000	8.33 ± 0.58	7.67 ± 0.58	11.00 ± 1.00	0.00
	500	6.67 ± 0.58	0.00	8.33 ± 0.58	0.00
	250	0.00	0.00	6.33 ± 0.58	0.00
	125	0.00	0.00	6.33 ± 0.58	0.00
E. camaldulensis bark ButE	4000	20.00 ± 1.00	16.33 ± 0.58	8.00 ± 1.00	10.33 ± 0.58
	2000	15.00 ± 1.00	12.67 ± 0.58	8.17 ± 0.76	9.00 ± 1.00
	1000	13.50 ± 0.50	10.50 ± 0.50	7.33 ± 0.76	8.50 ± 0.50
	500	11.00 ± 1.00	8.67 ± 0.58	7.33 ± 1.53	8.33 ± 1.15
	250	7.67 ± 0.58	6.67 ± 0.58	0.00	0.00
	125	7.67 ± 0.58	0.00	0.00	0.00
Standard antibiotic and negative controls					
Gentamicin		14	13	20	18
Dimethyl sulfoxide[b]		0.00	0.00	0.00	0.00

[a] The Inhibition zones values are presented as mean of three measurements without including the disc diameter. Inhibition > 15 mm (strong inhibition), 15–10 mm (moderate), and < 10 mm (weak). The 0.00 values meaning that the extract was not active.
[b] Negative control, discs were loaded with 10% of dimethyl sulfoxide.

minor concentrations in ButE of *E. camaldulensis* bark.

The EO of *C. dioscoridis* aerial parts components are given in Table 7. The main chemical constitutes are *Z*-(13,14-epoxy)tetradec-11-en-1-ol acetate (11.6%), γ-elemene (10.3%), tau.-muurolol (7.1%), cadina-3,9-diene (4.7%), widdrol hydroxyether (4.5%), lilial (4.4%), Guaia-1(10),11-diene (2.9%), 1,3-dioxolan-2-one, 5-methyl-4-(4,4-dimethyl-2,3-dimethylenecyclohexyl) (2.5%), caryophyllene oxide (2.5%), α-muurolene (2.45%), and 3-isopropyl-6,7-dimethyltricyclo [4.4.0.0(2,8)]decane-9,10-diol (2.3%).

4. Discussion

The evaluation of antibacterial impact of the studied extracts and essential oil against the growth of the isolated four phytopathogenic bacteria by means of IZs and MICs indicated that the activity ranged from strong to moderate inhibition. According to our survey about the research done on the antibacterial activity of natural extracts against the growth of plant bacterial pathogens especially on potato. The bark extracts of *Erythrina humeana* and *Delonix regia* exhibited weak and

Table 3
Minimum Inhibitory Concentrations (MICs) of extracts for antibacterial activity.

Extract	MIC value (μg/mL)			
	P. carotovorum	*R. solanacearum*	*Dickeya* spp.	*A. tumefaciens*
C. viminalis flower AcE	125	64	< 16	16
C. viminalis flower ButE	250	500	250	250
C. dioscoridis aerial parts EO	125	250	< 16	> 4000
E. camaldulensis bark ButE	16	125	500	250

Table 4
Profile of the chemical constitutes of the acetone extracts from *C. viminalis* flowers.

Compound name	Relative peak area	Molecular Formula	Molecular Weight	Standard index	Reverse standard index
4-Heptenal	4.62	$C_7H_{12}O$	112	661	791
5-Methyl furfural	4.34	$C_6H_6O_2$	110	778	926
2-Ethylcyclopentanone	1.87	$C_7H_{12}O$	112	607	860
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.31	$C_6H_8O_4$	144	575	781
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1.74	$C_6H_8O_4$	144	563	886
Phenol	2.48	C_6H_6O	94	773	883
γ-Heptalactone	3.61	$C_8H_{10}O$	122	631	714
β-Terpinyl acetate	5.11	$C_{12}H_{20}O_2$	196	823	883
α-Terpinolen	2.44	$C_{10}H_{16}$	136	821	859
1-Naphthol	1.13	$C_{10}H_8O$	144	637	840
(±)-*α*,*α*,4-trimethylcyclohex-3-ene-1-methanol	1.15	$C_{10}H_{18}O$	154	749	808
5-hydroxymethylfurfural	20.64	$C_6H_6O_3$	126	697	806
Pyrogallol	16.30	$C_6H_6O_3$	126	866	915
Ledol	2.79	$C_{15}H_{26}O$	222	815	837
Myristic acid	1.14	$C_{14}H_{28}O_2$	228	793	801
Bicyclo[2.2.1]heptane-1-methanol,7,7-ethylenedioxy-	4.33	$C_{10}H_{16}O_3$	184	681	715
Aspidinol	3.16	$C_{12}H_{16}O_4$	224	752	803
Dimethyl dodecanedioate	0.93	$C_{14}H_{26}O_4$	258	667	705
Palmitic acid	18.57	$C_{16}H_{32}O_2$	256	885	893
2-Methyl-4-isovalerylphloroglucinol	2.36	$C_{12}H_{16}O_4$	224	685	876

Table 5
Profile of the chemical constitutes of the *n*-butanol extract from *C. viminalis* flower.

Compound name	Area %	Molecular Formula	Molecular Weight	Standard index	Reverse standard index
Ethyl citral	0.36	$C_{11}H_{18}O$	166	670	699
(*Z*)-2-Pentenal	2.31	C_5H_8O	84	747	889
2-Hydroxymyristic acid	0.44	$C_{14}H_{28}O_3$	244	610	666
Eucalyptol	1.09	$C_{10}H_{18}O$	154	743	826
1-Butoxy-1-methoxybutane	2.53	$C_9H_{20}O_2$	160	811	984
Diglycerol	0.98	$C_6H_{14}O_5$	166	710	746
2-(*N*-Methylindole-3-yl)acetic acid	1.11	$C_{11}H_{11}NO_2$	189	660	815
2-Methoxynaphthalene	0.57	$C_{11}H_{10}O$	158	623	687
5-Hydroxymethylfurfural	7.19	$C_6H_6O_3$	126	882	920
1,1-Dibutoxybutane	4.17	$C_{12}H_{26}O_2$	202	839	862
1,4-Dioxaspiro[4.5]dec-7-ylmethanol	0.37	$C_9H1_6O_3$	172	658	715
Orthoformic acid triisobutyl ester	1.64	$C_{13}H_{28}O_3$	232	709	937
Dibutyl oxalate	1.84	$C_{10}H_{18}O_4$	202	697	871
4,5-Dimethyl-2-pentadecyl-1,3-dioxolane	1.26	$C_{20}H_{40}O_2$	312	684	811
2-Hydroxymyristic acid	9.41	$C_{14}H_{28}O_3$	244	657	662
4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester	1.32	$C_{11}H_{20}O_3$	200	625	700
Dibutyl Succinate		$C_{12}H_{22}O_4$	230	704	832
2,4,6-Trimethylmandelic acid	0.71	$C_{11}H_{14}O_3$	194	652	679
Palmitic acid	36.39	$C_{16}H_{32}O_2$	256	836	848
4-O-*α*-D-galactopyranosyl-α-D-glucopyranose	5.86	$C_{12}H_{22}O_{11}$	342	717	726
Desulphosinigrin	5.63	$C_{10}H_{17}NO_6S$	279	718	751
Shikimic acid	6.63	$C_7H_{10}O_5$	174	762	795
Aspidinol	0.55	$C_{12}H_{16}O_4$	224	707	776
Methyl isopalmitate	0.47	$C_{17}H_{34}O_2$	270	726	731
2-Methyl-4-isovalerylphloroglucinol	2.36	$C_{12}H_{16}O_4$	224	772	891

moderate activity, respectively, against the growth of *Pectobacterium* and *Dickeya* spp. [4]. Extracts of *Salvadora persica* (leaf, branch, and root-wood) demonstrated good antibacterial activities against the growth of *P. carotovorum* subsp. *carotovorum*, *D. solani*, *R. solanacerum*, *Enterobacter cloacae*, and *Bacillus pumilus* [44]. Wood and bark extracts of *Picea abies* and *Larix decidua* have been reported to have a moderate activity against the growth of some *Pectobacterium* and *Dickeya* spp. [45]. Our previous study reported that the extracts from branches of *C. viminalis* did not show any activity against the growth of *Dickeya* and *Pectobacterium* spp. [5].

The antibacterial activity of the studied extracts could be related to the presence of bioactive compounds in their extracts. *C. viminalis*

Table 6
Profile of the chemical constitutes of the *n*-butanol extract from *E. camaldulensis* bark.

Compound name	Area %	Molecular Formula	Molecular Weight	Standard index	Reverse standard index
α-Pinene	0.45	$C_{10}H_{16}$	136	758	765
Limonene	0.66	$C_{10}H_{16}$	136	745	777
1,8-Cineole (Eucalyptol)	8.19	$C_{10}H_{18}O$	154	769	777
Camphor	30.9	$C_{10}H_{16}O$	152	801	836
Menthol	8.87	$C_{10}H_{20}O$	156	774	828
8-Nonynoic acid methyl ester	45.63	$C_{10}H_{16}O_2$	168	751	781
Isobornyl acetate	2.64	$C_{12}H_{20}O_2$	196	809	847
Valeric acid, 3-tetradecyl ester	0.41	$C_{19}H_{38}O_2$	298	790	805
Docosane	0.48	$C_{22}H_{46}$	310	669	991
Pentacosane	0.47	$C_{25}H_{52}$	352	770	797
3-Octylundecyl-benzene	0.43	$C_{25}H_4$	344	757	822
8-(Methoxycarbonyl)octyl6-O-benzoyl-3,4-O-(1′,2′-dimethoxycyclohexane-1′,2′-diyl)-α,D-mannopyranoside	0.87	$C_{31}H_{46}O_{11}$	594	754	780

Table 7
Profile of the chemical constitutes of essential oil from *C. dioscoridis* aerial parts.

Compound name	Area %	Molecular Formula	Molecular Weight	Standard index	Reverse standard index
Caryophyllene-(I1)	1.72	$C_{15}H_{24}$	204	758	765
Cadina-3,9-diene	4.77	$C_{15}H_{24}$	204	745	777
Lilial	4.42	$C_{14}H_{20}O$	204	739	745
Guaia-1(10),11-diene	2.89	$C_{15}H_{24}$	204	769	777
α-Muurolene	2.45	$C_{15}H_{24}$	204	756	785
Germacrene D-4-ol	0.68	$C_{15}H_{26}O$	222	801	836
γ-Cadinene	2.05	$C_{15}H_{24}$	204	786	798
2-Isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene	1.32	$C_{15}H_{24}$	204	759	769
Hedycaryol	2.04	$C_{15}H_{26}O$	222	774	828
Caryophyllene oxide	2.48	$C_{15}H_{24}O$	220	758	787
Aromadendrene oxide-(2)	1.62	$C_{15}H_{24}O$	220	751	781
Aristolene epoxide	0.72	$C_{15}H_{24}O$	220	820	900
Cubenol	1.36	$C_{15}H_{26}O$	222	809	847
(+)-Ledene	1.44	$C_{15}H_{24}$	204	790	805
1,3-Dioxolan-2-one, 5-methyl-4-(4,4-dimethyl-2,3-dimethylenecyclohexyl)	2.49	$C_{14}H_{20}O_3$	236	669	991
tau.-Muurolol	7.14	$C_{15}H_{26}O$	222	647	959
γ-Elemene	10.29	$C_{15}H_{24}$	204	644	899
α-Cadinol	7.29	$C_{15}H_{26}O$	222	629	813
Carotol	1.04	$C_{15}H_{26}O$	222	770	797
3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)]decane-9,10-diol	2.27	$C_{15}H_{26}O_2$	238	767	828
Tricyclo[6.3.1.0(1,5)]dodecan-9-ol, 2-benzoyloxy-4,4,8-trimethyl-	1.27	$C_{22}H_{30}O_3$	342	757	761
E,E-6,8-Tridecadien-2-ol, acetate	1.76	$C_{15}H_{26}O_2$	238	754	781
2,6,10-Dodecatrien-1-ol, 12-acetoxy-2,6,10-trimethyl-, (E,E,E)-	1.79	$C_{17}H_{28}O_3$	280	757	822
Methyl hinokiate	0.92	$C_{16}H_{24}O_2$	248	756	784
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	11.62	$C_{16}H_{28}O_3$	268	754	780
Hexahydrofarnesyl acetone	1.85	$C_{18}H_{36}O$	268	720	947
Limonen-6-ol, pivalate	0.71	$C_{15}H_{24}O_2$	236	830	940
trans-Longipinocarveol	1.95	$C_{15}H_{24}O$	220	742	953
α-Cedren-9-β-ol	1.65	$C_{15}H_{24}O$	220	743	962
Alloaromadendrene oxide-(1)	1.28	$C_{15}H_{24}O$	220	757	961
2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	2.05	$C_{13}H_{22}O_3$	226	778	926
γ-Gurjunenepoxide-(1)	1.96	$C_{15}H_{24}O$	220	776	919
Nerolidol isobutyrate	1.02	$C_{19}H_{32}O_2$	292	607	860
1-Benzoxirene-2,5-dione, 4-(3-oxobutyl)hexahydro-3,3,4-trimethyl	0.96	$C_{13}H_{18}O_4$	238	591	903
Widdrol hydroxyether	4.52	$C_{15}H_{26}O_2$	238	863	886
Phytol	1.73	$C_{20}H_{40}O$	296	771	881
Dehydroisophytol	0.71	$C_{20}H_{38}O$	294	773	883
Pentacosane	1.78	$C_{25}H_{52}$	352	631	714

flowers are rich in polyphenols, flavonoids, saponins, steroids, alkaloids and triterpenoids contents, which possess good antibacterial and antifungal activities [16,46,47]. The flower aqueous extract has also shown an antibacterial activity [14]. The crude extract of leaves and flowers together (aerial parts) *C. viminalis* showed promising activity against *Candida albicans*, *C. kefyr*, G^+ ve and G^- ve bacteria [48]. 5-hydroxymethylfurfural compound which was isolated from seeds extract of *Cassia fistula* [49,50], was reported to have a significant bioactivity against the growth of certain pathogenic bacteria. Additionally, it was observed that at different concentrations (15, 20 and 25%) of 5-hydroxymethylfurfural it exposed a bactericidal activity against the growth of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* with different degrees [51].

Under certain conditions, the methanolic extract of *P. dioscoridis* from aerial parts was revealed significant antinociceptive effect on mice [52] and antidiarrheal activity on rabbits [53]. It was reported that the essential oil of *C. dioscoridis* exhibited promising antimicrobial activities against some tested micro-organisms [54]. Pyrogallol was found in leaves of *Mangifera indica* [55] and in the latex of *Holigarna grahamii* [56] as a bioactive compound. *Z*-[13,14-epoxy]tetradec-11-en-1-ol acetate which is the main compound in the aerial parts EO of *C. dioscoridis* with percentage of 11.6%, was found in the ethanol extract of

shed dried leaves powder of *Indigofera suffruticosa* [57].

The major compounds; α-gurjunene (10.8%), isocomene, δ-cadinene, *P*-gurjunene and 6-epi-shyobunol were identified in the EO of *C. dioscoridis* [22]. The following compounds have also been isolated and identified form the extracts of *C. dioscoridis* aerial parts kaempferol 3-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside, rutin, 5, 7, 4'-trihydroxy 8-C-glucopyranoside (vitexin), 5,7,4'-trihydroxy-6-Cglucopyranoside (isovitexin), quercetin-3-O-α-L-rhamnopyranoside, kaempferol 3-O-β-D-glucopyranosyl quercetin 3-O-β-Dglucopyranosyl, kaempferol, quercetin, caffiec acid, cholesterol, β-sitosterol, α-amyrin, conyzin, lupeol acetate, β-sitosterol glucoside, gallic acid, syringic acid, quercitin and chlorogenic acid [22,58,59].

Moreover, it is suggested that the occurrence of active compounds like1,8-cineole in Myrtaceae family (*Eucalyptus* and *Callistemon*) are well-known for their biological activities such as antibacterial, antioxidant, and antifungal activities [10,16,19,20,46,60].

5. Conclusion

In the present study, the antibacterial activity of extracts from *C. viminalis* (flowers), essential oil from *C. dioscoridis* (aerial parts), and ButE of *E. camaldulensis* (bark) was evaluated against *R. solanacearum*, *P. carotovorum* subsp. *carotovorum*, *A. tumefaciens* and *Dickeya* spp. The highest activity against *P. carotovorum* was found by *E. camaldulensis* bark ButE, *R. solanacearum* by *C. viminalis* flower AcE, *Dickeya* spp. by *C. viminalis* flower AcE and *C. dioscoridis* aerial parts EO, and *A. tumefaciens* by *C. viminalis* flower AcE. The main compounds in *C. viminalis* flowers AcE were 5-hydroxymethylfurfural, palmitic acid, and pyrogallol; in *C. viminalis* flower ButE were palmitic acid, 2-hydroxymyristic acid, 5-hydroxymethylfurfural, and shikimic acid; in *E. camaldulensis* bark ButE were 8-nonynoic acid methyl ester, camphor, menthol, and 1,8-cineole (eucalyptol), and in the EO of *C. dioscoridis* aerial parts were *Z*-(13,14-epoxy)tetradec-11-en-1-ol acetate, γ-elemene, *tau.*-muurolol, cadina-3,9-diene, widdrol hydroxyether, lilial, and guaia-1(10),11-diene (2.89%). It can be concluded that the studied extracts and essential oil resulted in strong to moderate antibacterial activity against the studied plant pathogens.

Acknowledgments

Authors would like to thank Dr. Mamoun S. M. Abd El-Kareem (Atomic and Molecular Physics Unit of the Experimental Nuclear Physics Department at the Nuclear Research Centre of the Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt), and Prof. Dr. Wafaa A. Mohamed (Conservation Department, Faculty of Archaeology, Cairo University, Giza 12613, Egypt) for their sincere help.

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