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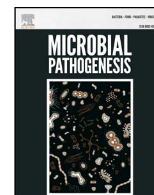

Effectiveness of root-bark extract from *Salvadora persica* against the growth of certain molecularly identified pathogenic bacteria

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ABSTRACT

The acetone extract from root-bark of *Salvadora persica* L. (Salvadoraceae), is assayed for its antibacterial activity against some bacterial pathogens. By GC/MS analysis, the main chemical components of the acetone extract were found to be benzylisothiocyanate (39.4%), and benzyl nitrile (benzeneacetonitrile) (37.9%). According the extract concentrations used, the measured inhibition zones observed were between from 13.6 to 18.6 mm, 15.3–23 mm, 13.3–18.3 mm, 13.3–18.3 mm, and 12.3–19 mm, against the isolated plant bacterial pathogens namely *Agrobacterium tumefaciens*, *Pectobacterium atrosepticum*, *Enterobacter cloacae*, *Dickeya solani* and *Ralstonia solanacearum*, respectively, whilst it was between 8 and 12 mm, 8–9.6 mm, 8–11.6 mm, and 8–10.3 mm against *Bacillus subtilis*, *Sarcina lutea*, *Escherichia coli* and *Staphylococcus aureus*, respectively. The minimum inhibitory concentration values of the extract were between 16 and 32 μg/mL against the growth of plant bacterial, and from 1000 to 2000 μg/mL against the growth of the human bacteria. In conclusion, the acetone extract of root-bark of *S. persica* showed strong antibacterial activity against the plant pathogens and some activity against the human pathogens were reported. The results suggested that using the acetone extract from root-bark of *S. persica* as bioactive agent against the growth of the studied plant bacterial pathogens.

1. Introduction

Bacteria belonging to the *Pectobacterium*, *Dickeya* genera and recently *Enterobacter cloacae* are causal agents of blackleg and tuber soft rot of potato [1,2]. In seed potato production, these diseases are next in economic importance to bacterial wilt caused by *Ralstonia solanacearum* [3]. *Agrobacterium tumefaciens* is the causal agent of crown gall disease; the common neoplastic disease of dicot plants, including many woody shrubs and various herbaceous plants including mainly stone and pome fruit-trees, grapevines, roses and ornamental plants [4]. The polymerase chain reaction (PCR) allows biologists to sequence DNA from many species or individuals [5], hence, the use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been by far the most common housekeeping genetic marker used [6]. *recA* gene thought to be universally present in prokaryotic and eukaryotic cells as it shows a high degree of sequence conservation and his sequence comparisons have been used speculate about phylogenetic relationships among genera and species belonging to the former *Erwinia* genus [7,8].

Bacterial pathogens of *Bacillus cereus* (is one of the major food-borne pathogenic bacteria and a common contaminant of food and dairy products), *Staphylococcus aureus* (responsible for infections of three general types: superficial, life threatening systemic, and toxins, including food poisoning toxic shock), *Serratia lutea* (causes skin infections in those with weak immune systems), and *Echerichia coli* (is linked to diseases in just about every other part of the body such as pneumonia, meningitis, and traveler's diarrhea) have been documented for thier pathogenicity [9].

Extracts from Miswak (*Salvadora persica*), have been widely used in toothbrushes for the prevention of tooth decay [10]. The antimicrobial agents extracted from leaves, stem, seeds, and roots were used to treat various oral bacteria [11], leucoderma, some skin diseases, joint pain and toothache [12–15]. Different solvents were used to extract the antimicrobial against from *Salvadora persica*, for example, aqueous [16], alcoholic [10,17], methanol:*n*-hexane [18], and diluted acetone [19].

Methanol:*n*-hexane of root-wood showed moderate activity against the potato bacterial pathogens *Pectobacterium carotovorum* subsp. *carotovorum*, *Dickeya solani*, *Ralstonia solanacerum*, *Enterobacter cloacae*, and *Bacillus pumilus* [18], good bioactivity was found against the growth of *Candida albicans*, *C. glabrata*, and *C. parapsilosis* strains when

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the diluted acetone extract from stem was applied [19]. The leaf extract observed good antibacterial activity against *Staphylococcus aureus* [20]. In-vivo the extracts showed moderate antimicrobial activity against lactobacilli and *streptococcus mutans* [21].

Furthermore, different chemical components have been isolated with different solvents and parts of the plant, *i.e.,* β-sitosterol in root extract [22], salvadoricine from leaf extract [23], pyrrolidine, and pyrrole in sticks [24], kaempferol 3-α-L-rhamnosyl-7-β-xylopyranoside, quercetin, and kaempferol from the stem extract [25], benzyl isothiocyanate in root oil [26], fatty acid ethyl esters from roots and stems [27], benzylnitrile from the leaf oil [11], β-sitosterol and stigmasterol in stem extract [10], *N*-benzylbenzamide (branch extract), 2,6-dimethyl-*N*-(2-methyl-α-phenylbenzyl)aniline (leaf extract) and benzeneacetonitrile (root-wood extract) [18].

This study is generally designed to evaluate the antibacterial activity of acetone extract from root-bark of *S. persica* against the growth of nine pathogenic bacteria. The chemical composition of the extract was performed using gas chromatography–mass spectrometry (GC/MS).

2. Material and methods

2.1. Plant material and extraction

Root samples of *Salvadora persica* were supplied by Dr. Hayssam M. Ali (Botany and Microbiology Department of the College of Science at King Saud University) on July 2017 to the Forestry and Wood Technology Department (Faculty of Agriculture at Alexandria University). The plant was previously authenticated with the voucher number Zidan0043 [18]. The roots were air-dried to reach the moisture content of 10.12% at the laboratory conditions for two weeks and the bark was separated from the roots and ground to powder. The powder of root-bark was extracted by soaking method, whilst sample of 100 g was soaked in 150 mL of acetone for one week and the extraction was repeated three times every two days in the week (Fig. 1).

The extract was filtrated throughout cotton plug and the filter paper (Whatman no. 1), then the solvent was evaporated under reduced pressure using a rotary evaporator to concentrate the extract. The quantity of extract was 5.15 g/100 g air-dry sample. The acetone root-bark extract was stored for one day at 4 °C prior to chemical and bioassay analyses. Acetone extract of *S. persica* root-bark was prepared at the concentrations of 125, 250, 500, 1000, 2000, 3500 and 7000 μg/mL, by diluting it in 10% dimethylsulfoxide (prepared with distilled water) [18].

2.2. Isolation of phytopathogenic bacteria from plant material

Most methods used for identification of wilts, brown rot, soft rot, blackleg and tumors (Fig. 2) causing by bacteria require isolation of viable cells from samples and growth and purification of the bacteria prior to analyses. From symptomatic tissues, it is best to sample from the advancing front of the rot/galls or from newly diseased tissue to avoid interference and growth suppression by contaminating saprophytes. The sample is usually suspended and diluted in sterile water or buffer and a loopful streaked on a growth medium selective for Enterobacteriaceae, Burkholderiaceae and Rhizobiaceae pathogens [28,29]. To protect bacterial cells from oxidative stress due to the release of the plant compounds during tissue preparation or enrichment, an antioxidant 0.05%- dithiothreitol- is commonly used [29,30]. Plates are incubated at different temperatures as the pathogens have different optimal growth temperatures [31]. Depending on the medium, bacterial colonies appear after 24–48 h at 21–37 °C.

2.3. Morphological and biochemical characteristics of the isolated bacteria

The morphological and biochemical characteristics of the isolated bacteria were studied by performing the standard tests recommended [32–34].

2.4. Molecular detection methods

2.4.1. DNA extraction

After growth of the strains on nutrient glycerol medium purified colonies picked, subjected to DNA extraction using the G-spin genomic DNA isolation kit (iN-tRON Biotechnology, Seongnam, Korea) and then stored at −20 °C until needed.

2.4.2. Identification of bacteria using conserved gene sequence analysis

A fragment of 16S rRNA gene was amplified with primers p0-f (GAAGAGTTTGATCCTGGCTCAG) and p6-r (CTACGGCTACCTTGTTA CGA) [2] and recA gene was amplified with the recA-specific primers recA_f (GGTAAAGGGTCTATCATGCG) and recA_r (CCTTCACCATACAT AATTTGGA) [7]. The PCR products obtained were sequenced by Macrogen, Inc. (Korea). The strains were identified based on a comparison of their nucleotide sequences with those in the GenBank database using the BLAST software on the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/).

2.5. Antibacterial activity assay

The antibacterial activity of the acetone extract from root-bark of *S. persica* was assayed by disk diffusion method [35] against the growth of selected plant pathogenic bacteria; *Agrobacterium tumefaciens*, *Pectobacterium atrosepticum*, *Enterobacter cloacae*, *Dickeya solani* and *Ralstonia solanacearum* as well as human pathogenic bacteria; *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538. Freshly 24-h's old bacterial suspension (1.0×10^5 CFU/mL) were spread over the Mueller Hinton Agar-media in Petri dishes, then sterile discs (Whatman no. 1) with dimeter of 5 mm were put over the media. The discs were loaded with 50 μL of the acetone extract with 125, 250, 500, 1000, 2000, 3500 and 7000 μg/

Fig. 1. Root-bark of *Salvadora persica* (Miswak) and its acetone extract.

Fig. 2. Naturally infected plant samples symptoms, A; *Ralstonia solanacearum* brown rot on potato tuber B; *Pectobacterium atrosepticum* on potato stems, C; *Enterobacter cloacae* soft rot on potato, D; *Dickeya solani* on potato stems,E; *Agrobacterium tumefaciens* tumor on olive. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mL. After 24 of incubation at 37 °C for human bacterial pathogens and at 30 °C for plant pathogens, the inhibition zones (IZs) around the discs were measured in millimeters using a ruler. Controls discs with negative imethylsulfoxide and positive (Tobramycin 10 μg/disc) were performed, and all tests were measured in triplicate [36]. Minimum inhibitory concentrations (MICs) was performed in 96-well micro-plates [37] using serial dilutions of the acetone extract ranged between 4 and 4000 μg/mL.

2.6. GC/MS analysis of acetone extract

The chemical constituents of acetone extract was analyzed by a Trace 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 GC/MS was located at the Atomic and Molecular Physics Unit of the Experimental Nuclear Physics Department at the Nuclear Research Center of the Egyptian Atomic Energy Authority (Inshas, Cairo, Egypt). The oven temperature program was set as follows: 45–165 °C (4 °C/min) then 165–280 °C (15 °C/min) with post run off at 280 °C. Samples (1 μL) were injected at 250 °C, with split/split-less injector (50:1 split ratio) in the split-less mode flow at 10 mL/min. The injector and detector (MS transfer line) temperatures were kept at 250 °C. Helium used as the carrier gas (flow rate 1 mL/min), was kept in constant flow rate of 1 mL/min. The solvent delay was 2 min, and diluted samples of 1 μL were injected automatically using an Auto-sampler AS3000 coupled with the GC unit in the split mode. The MS detector was operated in electron impact ionization (70 eV) scanning from m/z range of 40–550 in full scan mode. The components were identified by comparison of their retention times and mass spectra with those of the WILEY 09 and NIST 11 mass spectral database [38].

2.7. Statistical analysis

Data of inhibition zones were analyzed using Analysis of variance- in completely randomized design and were reported as mean ± standard division- using SAS software version 8.2 [39]. The significant difference among the concentrations was measured with the criterion of $p = 0.05$.

3. Results

3.1. Isolation trails and identification

All the isolation trails and morphological and biochemical identification (Table 1) as expected revealed that all tested strains caused wilt, brown rot, severe soft rot, black leg and galls diseases belong to *Ralstonia solanacearum*, *Enterobacter cloacae*, *Dickeya solani*, *Pectobacterium atrosepticum* and *Agrobacterium tumefaciens*.

3.2. Partial sequencing of the 16S rRNA and rec A genes of the studied strains

After BLAST comparison, all the partial sequences of the 16S rRNA and *rec* A genes confirmed the biochemical identification of all strains tested. These partial sequences were deposited in GenBank database, and the accession numbers are illustrated in (Table 2).

3.3. Antibacterial activity

Results of the inhibition zones (mm) observed by applying the acetone extract from root-bark of *S. persica* against the growth of plant pathogens; *A. tumefaciens*, *P. atrosepticum*, *E. cloacae*, *D. solani* and *R. solanacearum* (Table 3) and human pathogens; *B. subtilis*, *S. lutea*, *E. coli* and *S. aureus* (Table 4) were reported. By increasing the concertation of the extract, the IZs value increased.

In Table 3, the highest values of IZs observed against the growth of *A. tumefaciens* were 18.3 mm followed with 18.6 mm by applying the extract at 3500 and 7000 μg/mL and those values are higher than from the Tobramycin (17.6 mm).

All the concentrations used 125, 250, 500, 1000, 2000, 3500 and 7000 μg/mL from the extract are showed IZ values of 15.3 mm, 15.3 mm, 18.6 mm, 20.3 mm, 20.3 mm, 21.3 mm and 23 mm, respectively, against the growth of *P. atrosepticum*, which were higher than the value reported by the antibiotic used (Tobramycin 14.3 mm).

At the concentrations of 3500 and 7000 μg/mL, the acetone extract of *S. persica* root-bark showed the highest IZs values of 17.3 mm, and 18.3 mm, respectively, against the growth of *E. cloacae*, which equal or higher than those from Tobramycin (17.6 mm).

The highest activity against the growth of *Dickeya solani* were reported by applying the extract at 1000, 2000, 3500, and 7000 μg/mL, where the IZs were 16.3 mm, 16 mm, 17.3 mm, and 18.3 mm, respectively, and reported to be higher than the value of Tobramycin (15.3 mm).

Also, against the growth of *R. solanacearum*, all the concentrations used 250, 500, 1000, 2000, 3500 and 7000 μg/mL were observed good activity with IZ values of 13.3 mm, 16.3 mm, 16.3 mm, 18.3 mm, 18.6 mm, and 19 mm, respectively, which are higher than the value of Tobramycin (12.6 mm).

Table 4 showed that the highest activity against the growth of *B. subtilis* was observed at the concentration of 7000 μg/mL with IZ value of 12 mm, which lower than the value of Tobramycin (15.3 mm). Some activity was observed against *S. lutea*, whilst the highest activity was observed with IZ value 9.6 mm at the concertation of 7000 μg/mL. Also, some activity was observed against *E. coli* and *S. aureus* with IZs value of 11.6 mm and 10.3, respectively, at the concentration of 7000 μg/mL.

The value of IZs observed against the growth of the human pathogenic bacteria were not promised even the values were higher than those reported by the antibiotic used. It showed notice that the

Table 1
Morphological traits, physiological and biochemical reactions of all isolates obtained from diseased samples under this study.

Bacterial isolate	Characteristic										
	Shape(rods)	Gram staining	Motility	Anaerobic growth	Potato soft rot	Growth at 4 °C	Growth at 40 °C	Growth at 37°C	Gelatin liquefaction	Mucoid growth	Kovac's oxidase
Ralstonia solanacearum	+	-	+	+	-	-	-	+	-	+	+
Pectobacterium atrosepticum	+	-	+	+	+	n	+	+	+	+	-
Enterobacter cloacae	+	-	+	+	+	n	+	+	+	+	-
Dickeya solani	+	-	+	+	+	n	-	+	+	+	-
Agrobacterium tumefaciens	+	-	+	+	-	n	+	+	n	+	-

Bacterial isolate	Characteristic										
	H_2s from cysteine	Indole production	Levan production	3-ketolactose production	R. substance from sucrose	Arginine dihydrolyase	Urease production	Growth in 5% NaCl	Sensitivity to erythromycin	Phosphatase	Malonate utilization
Ralstonia solanacearum	n	n	-	n	-	-	-	-	-	-	n
Pectobacterium atrosepticum	+	-	-	n	-	n	-	-	+	-	-
Enterobacter cloacae	+	-	-	n	-	n	-	+	-	-	+
Dickeya solani	+	+	-	n	-	n	-	-	+	+	+
Agrobacterium tumefaciens	n	n	-	+	-	n	-	+	-	-	n

Bacterial isolate	Characteristic										
	Starch hydrolysis	Glucose	α-methyl glucoside	Maltose	Lactose	L-Arabinose	Dulcitol	Manitol	trehalose	erythritol	Alkali production tartrate
Ralstonia solanacearum	-	a	n	a	a	-	-	-	n	n	n
Pectobacterium atrosepticum	+	**a**	-	-	a	**a**	**a**	**a**	a	n	n
Enterobacter cloacae	+	a/g	a	a	a	a	a	a	a	n	n
Dickeya solani	+	a	-	a	a	a	a	a	-	n	n
Agrobacterium tumefaciens	+	a	n	a	n	n	a	a	n	-	-

+ = More than 80% of isolates gave positive reaction - = Less than 20% of isolates gave negative reaction, a = acid, g = gas and n = not determined.

Table 2
Accession numbers, isolate code and source of partial 16S rRNA and *rec* A genes of studied phytopathogenic bacterial isolates in the GenBank.

No.	Bacterial isolates	Isolate code	gene	source	Accession no.
1	*Ralstonia solanacearum*		16SrRNA	Dr. Said Behiry	GH425351
2	*Dickeya solani*	DCH11	*rec*A	Dr. Said Behiry	HF569035
3	*Enterobacter cloacae*	ENCL68	*rec*A	Dr. Said Behiry	HF569036
4	*Pectobacterium atrosepticum*		16SrRNA	This study	MG706146
5	*Agrobacterium tumefaciens*		16SrRNA	This study	MG706145

Tobramycin was not active against the growth of *S. lutea*, and *S. aureus*.

According to the MIC values of the extract against the studied bacteria (Table 5), the highest activities were reported against the plant bacterial pathogens with MIC values of 32, 16, 32, 32, and 16 μg/mL, against the growth of *A. tumefaciens*, *P. atrosepticum*, *E. cloacae*, *D. solani* and *R. solanacearum*, respectively. On the other hand the MIC values were not also promised against the growth of the human bacteria, which were 2000, 1000, 2000 and 1000 μg/mL, against the growth of *B. subtilis*, *S. lutea*, *E. coli* and *S. aureus*, respectively.

3.4. Chemical composition of extract

Table 6 presents the chemical composition of acetone extract from root-bark of *S. persica*. The main abundant constituents were benzylisothiocyanate (39.4%), benzyl nitrile or benzeneacetonitrile (37.9%), *N*-ethyl-2,4-dimethyl-3-tetrazol-1-yl-benzenesulfonamide (4.10%), stearic acid, 3-(octadecyloxy)propyl ester (3.6%), and 2-benzylhexahydropyrrolizin-3-one (2.72%). Furthermore, other nitrogen-based compounds such as 3,7-dibenzyl-1,5-dimethyl-3,7-diazabicyclo [3.3.1] nonan-9-ol, 2-({[4-(benzyloxy)phenoxy]acetyl}amino)benzoic acid, 2-benzyl-2-isopropenyl malononitrile, benzenemethanamine, and *N*-hydroxy-*N*-(phenylmethyl)-, were identified with minor amounts. The chemical structures of benzylisothiocyanate and benzyl nitrile are shown in Fig. 3.

4. Discussion

As the antibacterial activity observed that the inhibition zones against the studied human bacterial pathogens were nearly weak according to the classification shown in Table 4, but generally it was higher than the values of the used antibiotic. Furthermore, the extract showed promising activity against all the studied plant bacterial pathogens (Table 3). Different parts of *S. persica* plant extracts exhibited moderate activity against *R. solanacearum*, *E cloacae* and *D. solani* [18], also EL-Hefny et al. [40] found that n-hexane fruit extract of *Phytolacca dioica* and *Ziziphus spina-christi* exhibited strong antibacterial activity against *R. solanacerum* at concentration 1000 μg/mL with IZ value of 11.6 mm and 10 mm respectively, meanwhile, a satisfied moderate activity obtained from *Picea abies* and *Larix decidua* extracts (bark and wood) against the growth of *P. atrosepticum, P. carotovorum*, and *D. solani* [41]. Previous studies showed that the leaf extract showed IZs ranged from 10.5 mm to 31.5 mm against *Staphylococcus aureus* [20]. Roots and stems aqueous extracts have been proven a promising antimicrobial activity which relate to compounds have anionic (sulfate, chloride, thiocynate, and nitrate) [42]. Other studies reported that the compound benzyl isothiocyanate, had a highly bioactivity against gram-negative bacteria [28,43]. Strong antibacterial activity against *Streptococcus* sp. and *Staphylococcus aureus* was reported [44]. Additionally, the extract showed good antimicrobial effects on *S. mutans* and *E. faecalis* [45]. Pulp and bark extracts of *S. persica* showed significant effects as an antimicrobial agent [46]. *S. persica* extract also

Table 3
Antibacterial activity of acetone extracts from *Salvadora persica* root-bark against the growth of some plant pathogenic bacteria.

Extract Concentration μg/mL	Diameter of inhibition zones[a] (mm ± standard division)														
	Agrobacterium tumefaciens			*Pectobacterium atrosepticum*			*Enterobacter cloacae*			*Dickeya solani*			*Ralstonia solanacearum*		
	IZ	−95%	+95%	IZ	−95%	+95%	IZ	−95%	+95%	IZ	−95%	+95%	IZ	−95%	+95%
125	13.67 ± 0.58	12.23	15.10	15.33 ± 0.58	13.90	16.77	13.33 ± 0.58	11.90	14.77	13.33 ± 0.58	11.90	14.77	12.33 ± 0.58	10.90	13.77
250	14.33 ± 0.58	12.90	15.77	15.33 ± 0.58	13.90	16.77	14.67 ± 0.58	13.23	16.10	14.67 ± 0.58	13.23	16.10	13.33 ± 0.58	11.90	14.77
500	15.33 ± 0.58	13.90	16.77	18.67 ± 1.15	15.80	21.54	14.00	14.00	14.00	14.67 ± 1.15	11.80	17.54	16.33 ± 1.53	12.54	20.13
1000	16.33 ± 0.58	14.90	17.77	20.33 ± ± 0.58	18.90	21.77	15.33 ± 0.58	13.90	16.77	16.33 ± 1.53	12.54	20.13	16.33 ± 1.53	12.54	20.13
2000	16.33 ± 0.58	14.90	17.77	20.33 ± 0.58	18.90	21.77	16.00 ± 1.15	16.00	16.00	16.00 ± 1.00	13.52	18.48	18.33 ± 1.53	14.54	22.13
3500	18.33 ± 0.58	16.90	19.77	21.33 ± 1.15	18.46	24.20	17.33 ± 0.58	14.46	20.20	17.33 ± 1.15	14.46	20.20	18.67 ± 1.53	14.87	22.46
7000	18.67 ± 0.58	17.23	20.10	23.00	23.00	23.00	18.33 ± 2.08	13.16	23.50	18.33 ± 2.08	13.16	23.50	19.00 ± 1.00	16.52	21.48
Tobramycin (10 μg)[b]	17.67 ± 0.58			14.33 ± 1.15			17.67 ± 1.53			15.33 ± 1.53			12.67 ± 0.58		
Dimethyl sulfoxide[c]	0.00			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 negative control was not active.
[b] Positive control.
[c] Negative control, discs were loaded with 10% of dimethyl sulfoxide.

Table 4
Antibacterial activity of acetone extracts from *Salvadora persica* root-bark against the growth of some human pathogenic bacteria.

Extract Concentration μg/mL	Diameter of inhibition zones[a] (mm ± standard division)											
	Bacillus subtilis			*Sarcina lutea*			*Escherichia coli*			*Staphylococcus aureus*		
	IZ	−95%	+95%	IZ	−95%	+95%	IZ	−95%	+95%	IZ	−95%	+95%
125	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
250	8.00	8.00	8.00	8.33 ± 0.58	6.90	9.77	9.33 ± 0.58	7.90	10.77	8.00	8.00	8.00
500	9.00	9.00	9.00	8.00	8.00	8.00	9.67 ± 1.15	6.80	12.54	9.33 ± 0.58	7.90	10.77
1000	10.33 ± 0.58	8.90	11.77	8.67 ± 1.15	5.80	11.54	9.67 ± 0.58	8.23	11.10	9.00	9.00	9.00
2000	10.67 ± 0.58	9.23	12.10	8.67 ± 0.58	7.23	10.10	10.00	10.00	10.00	9.67 ± 0.58	8.23	11.10
3500	10.67 ± 0.58	9.23	12.10	8.33 ± 0.58	6.90	9.77	11.33 ± 0.58	9.90	12.77	9.67 ± 1.15	6.80	12.54
7000	12.00	12.00	12.00	9.67 ± 1.15	6.80	12.54	11.67 ± 0.58	10.23	13.10	10.33 ± 0.58	8.90	11.77
Tobramycin (10 μg)[b]	15.33 ± 0.58			0.00			6.00			0.00		
Dimethyl sulfoxide[c]	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 positive or negative controls were not active.
[b] Positive control.
[c] Negative control, discs were loaded with 10% of dimethyl sulfoxide.

Table 5
Minimum Inhibitory Concentrations (MICs) of *Salvadora persica* root-bark for antibacterial activity.

MIC value (μg/mL)								
Agrobacterium tumefaciens	*Pectobacterium atrosepticum*	*Enterobacter cloacae*	*Dickeya solani*	*Ralstonia solanacearum*	*Bacillus subtilis*	*Sarcina lutea*	*Escherichia coli*	*Staphylococcus aureus*
32	16	32	32	16	2000	1000	2000	1000

reported to have a selective inhibitory effect on the levels of certain bacteria [47].

The chemical analysis showed the presence of benzylisothiocyanate, benzyl nitrile (benzeneacetonitrile), as main compounds in the acetone extract of root-bark. Previously, benzyl nitrile was found as the main compound in the leaves essential oil of *S. persica* [11]. While benzylisothiocynate was found in root extracts [48,49] as well as salvadourea [50]. Oleic, linolic, and stearic as fatty acids in the form of esters were identified the crude extract of *S. persica* [27]. Recently, benzeneacetonitrile (71.47%) and benzylisothiocyanate (5.05%), were identified from the methanol:*n*-hexane extracts of the root-wood of *S. persica* [18]. Some isolated compounds such as butanediamide, *N1*,*N4*-bis(phenylmethyl)-2(s)-hydroxy-butanediamide, *N*-benzyl-2,*N*-benzyl-2-phenylacetamide, and *N*-benzylbenzamide were identified as benzylamides [51]. Root oil contains mainly of benzyl isothiocyanate, limonene and α-pinene [26]. Alkaloids, tannins and saponins were found in Root bark [52]. Furthermore, gammamonoclinic sulphur, benzyl glucosinolate,

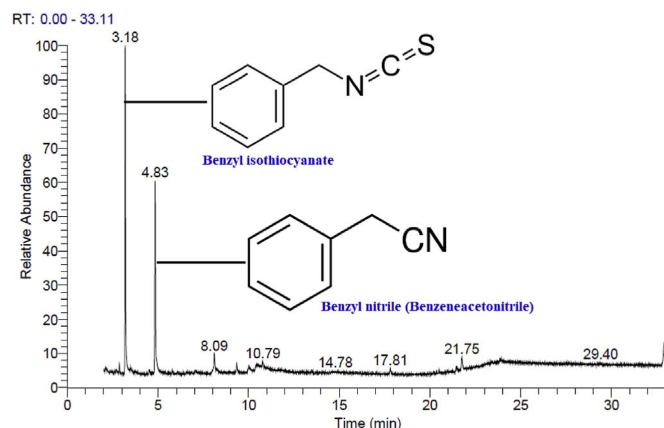

Fig. 3. GC/MS spectra of acetone extract from root-bark of *Salvadora persica* and the chemical structure of benzyl isothiocyanate and benzyl nitrile as main compounds.

Table 6
Chemical constitutes of the acetone extracts from *Salvadora persica* root-bark.

RT min.	Compound name	Relative peak area	Molecular Formula	Molecular Weight	Standard index	Reverse standard index
2.70	Benzaldehyde	0.81	C_7H_6O	106	829	830
2.86	Decane	0.97	$C_{10}H_{22}$	142	837	796
3.18	Benzyl isothiocyanate	39.48	C_8H_7NS	149	783	791
3.49	3,7-Dibenzyl-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-ol	0.70	$C_{23}H_{30}N_2O$	350	786	828
4.51	2-({[4-(benzyloxy)phenoxy]acetyl}amino)benzoic acid	0.93	$C_{22}H_{19}NO_5$	377	799	805
4.83	Benzyl nitrile (Benzeneacetonitrile)	37.93	C_8H_7N	117	794	800
5.77	2-Acetonyl-9-[3-deoxy-á-d-ribouranosyl]hypoxanthine	0.63	$C_{13}H_{16}N_4O_5$	308	794	807
9.33	2-Benzyl-2-isopropenyl malononitrile	1.87	$C_{13}H_{12}N_2$	196	788	803
10.02	*N*-Ethylbenzylamine	1.59	$C_9H_{13}N$	135	786	830
10.44	Stearic acid, 3-(octadecyloxy)propyl ester	3.62	$C_{39}H_{78}O_3$	594	838	842
10.78	1,7-Diphenyl-4-(3-phenylpropyl)heptane	1.60	$C_{28}H_{34}$	370	798	807
17.81	Benzenemethanamine, N-hydroxy-N-(phenylmethyl)-	1.07	$C_{14}H_{15}NO$	213	808	814
21.46	Eicosanoic acid	1.06	$C_{20}H_{40}O_2$	312	808	815
21.75	2-Benzylhexahydropyrrolizin-3-one	2.72	$C_{14}H_{17}NO$	215	805	812
32.92	*N*-ethyl-2,4-dimethyl-3-tetrazol-1-yl-benzenesulfonamide	4.10	$C_{11}H_{15}N_5O_2S$	281	808	816

salvadourea, m-anisic acid, sitosterol, benzyl isothiocyanate, and trimethylamine were presented to have a promising antiviral, antibacterial, antimycotic, antifungal, anti-parasitic [18,48].

5. Conclusion

According to the results shown in the present work, the acetone extract from root-bark of *S. persica* presented strong antibacterial activity against the isolated plant pathogens *A. tumefaciens*, *P. atrosepticum*, *E. cloacae*, *D. solani* and *R. solanacearum*, which causes many diseases to some economical crops. Also, the activity against the growth of the human bacteria was not promised even it was higher than the antibiotic used. The main components identified in the acetone extract of *S. persica* root-bark were benzylisothiocyanate, benzyl nitrile or benzeneacetonitrile, *N*-ethyl-2,4-dimethyl-3-tetrazol-1-yl-benzene-sulfonamide. We suggesting to use this extract in further applications against the plant pathogenic bacterial but in filed to generalize the results.

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