Chemical Composition and Bioactivity of *Salvadora persica* Extracts against Some Potato Bacterial Pathogens

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Potent antibacterial activities of solvent extracts (methanol:*n*-hexane) from the branch, leaf, and root-wood of *Salvadora persica* were examined against potato phytopathogenic bacteria, namely *Pectobacterium carotovorum subsp. carotovorum, Dickeya solani*, *Ralostonia solanacerum*, *Enterobacter cloacae*, and *Bacillus pumilus*. The main chemical constituents analyzed by gas chromatography–mass spectrometry (GC/MS) in the branch extracts were *N*-benzylbenzamide (71.08%), decane (3.17%), stigmasterol (3.17%), 9-desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol (2.33%), and *β*-sitosterol (2.15%). The main components in the leaf extracts were 2,6-dimethyl-*N*-(2-methyl-*α*-phenylbenzyl)aniline (28.65%), spiculesporic acid (13.60%), homo-*γ*-linolenic acid (12.63%), and methyl hexadecanoate (11.01%). The root-wood extracts contained, as primary parts, benzeneacetonitrile (71.47%), 4-aminocarbonyl-5-fluoro-1-*α*-D-ribofuranosyl-imidazole (10.99%), and benzylisothiocyanate (5.05%). The extracts from the root-wood showed moderate antibacterial activity against the potato bacterial pathogens, which was followed by leaf and branch extracts. The results suggested that *S. persica* plant extracts could be used as bioagents against potato soft and brown rot bacterial pathogens.

Keywords: Salvadora persica; Leaf; Root-Wood; Branch; Antibacterial activity; Chemical composition

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INTRODUCTION

Potato is an important vegetable crop in Egypt. Annually, approximately 4,800,000 tons are produced from approximately 178,000 hectares, which makes Egypt the top potato producer in Africa (FAO STAT 2013). Potato plants are subject to numerous pathogens and pests, which cause considerable quantitative and qualitative potato yield losses in Egypt. Such pathogenic problems are caused by bacterial diseases, especially brown rot caused by *Ralostonia solanacerum* (Yabuuchi *et al.* 1995) and soft rot and blackleg caused by *Pectobacterium carotovorum*, *Dickeya*, *Enterobacter,* and *Bacillus* species (Behiry 2013; Salem 2013; Ashmawy *et al.* 2014).

The first authenticated report of Brown rot disease in Egypt was in the last century (Sabet 1961), and Mickail *et al.* (1974) made the first survey on the organism. In

seed potato production, the contamination of seed tubers with soft rot bacteria (Pérombelon 2002; Toth *et al.* 2003), is one of the biggest problems, which causes blackleg, rotting of potato stems in the field, and soft rot of tubers during storage (Gardan *et al.* 2003; Laurila *et al.* 2008).

Salvadora persica (Miswak), which belongs to family Salvadoraceae, has been used in toothbrushes for the prevention of tooth decay (Arora and Kalia 2013). The leaf extracts act as an antibacterial agent to various oral bacteria (aerobic) with results comparable to known antibiotics (Alali and Al-Lafi 2003).

Several studies have reported that *S. persica* extracts and seed oil have great medicinal uses in the treatment of nose troubles, gonorrhea, leucoderma, scabies, scurvy, some skin diseases, joint pain and toothaches; it is also used as a laxative and as a general body tonic (Elvin-Lewis *et al.* 1980; Alali and Al-Lafi 2003; Darmani *et al.* 2003; Khalessi *et al.* 2004; Ahmed *et al.* 2008).

Leaf extracts of *S. persica* exhibit several pharmacological properties including carminative, antiseptic, antifungal, antibacterial, diuretic, analgesic, anthelmintic, astringent, hypoglycaemic, antiplasmodial, anticaries, antispasmodial, antiscorbutic, and anticonvulsant properties, as well as action against hepatic disorders (Al-Bagieh *et al.* 1994; Al-Bagieh and Almas 1997; Ali *et al.* 2002; Almas *et al.* 2005; Saini *et al.* 2006; Paliwal *et al.* 2007). Extracts of stems have antiplaque (Chawla 1983) and antimicrobial activities (Almas 2001). Aqueous extracts are more effective than methanol extracts against some pathogenic bacteria (Al-Bayati and Sulaiman 2008); however, Al-Bagieh and Almas (1997) showed that alcoholic extracts have more potent antimicrobial activity than aqueous extracts.

The heterogeneous components extracted from *S. persica* have been reported to have antimicrobial activities (Akhtar *et al.* 2011). Pulp and bark extracts show significant differences in their antimicrobial activities (Almas and Al-Bagieh 1999). *S. persica* extract (20%) is effective as an antifungal and antibacterial agent against *Candida albicans* and *Enterococcus faecalis* (Al-Obaida *et al.* 2010). The diluted acetone extract of dry stems (300 mg/mL) shows good inhibitory activity against *C. albicans*, *C. glabrata*, and *C. parapsilosis* strains with inhibition zones (IZs) that range from 10.33 mm to 15 mm (Noumi *et al.* 2010).

Volatile oils extracted from the roots and stems of *S. persica* contain fatty and other organic acid ethyl esters (Abdelrahman *et al.* 2003). Aqueous extracts of the roots contain chlorine, trimethylamine, and sulphur compounds with antimycotic activity (Al-Otaibi and Angmar 2004). Benzylisothiocyanate is the main component in root oil (Bader *et al.* 2002), which has good activity against Herpes simplex virus, *Streptococcus mutans*, and *Candida albicans* (Al-Bagieh 1992, 1998; Al-Bagieh and Weinberg 1988). *β*-Sitosterol has been found in the roots of *S. persica* (Ezmirly *et al.* 1978). The zone of inhibition against the growth of *Staphylococcus aureus* ranges from 10.5 mm to 31.5 mm for the leaf extract of *S. persica* and the combination of tetracycline with the stem extract of *S. persica*, respectively (Ahmed *et al.* 2010).

Salvadoricine, an indole alkaloid, has been isolated from *S. persica* leaves (Malik *et al.* 1987). Volatile oils from the leaves contain benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and *β*-caryophyllene (Alali and AL-Lafi 2003). Identified flavanoids and flavanoid glycosides include kaempferol 3-*α*-L-rhamnosyl-7-*β*-xylopyranoside, quercetin, and kaempferol (Kamil *et al.* 2000).

Ethanolic extracts of the stems contain *β*-sitosterol, stigmasterol, and *β*-sitosterol-D-glucoside (Arora and Kalia 2013). A sulfated glycoside, salvadoside (sodium 1-*O*-

benzyl-*β*-D-glucopyranoside-2-sulfate), was isolated from *S. persica* (Ohtani *et al.* 1992). Pyrrolidine, pyrrole, and piperidine derivatives have been identified in *S. persica* sticks (Galletti *et al.* 1993). Salvadoside and salvadoraside, which are glycoside compounds, have been reported in stem extracts (Kamel *et al.* 1992). Benzylisothiocyanate, saponins, tannins, resin, trimethylamine, and alkaloid have been isolated from the roots (El-Mostehy *et al.* 1983). *β*-Sitosterol, manisic acid, and salvadourea [1,3-bis-(3-methoxy-benzyl)-urea] were isolated from the root by Ray *et al.* (1975). 2-Furancarboxaldehyde-5-(hydroxymethyl), furan-2-carboxylic acid-3-methyl- trimethylsilyl ester, and D-erythro-pentofuranose-2-deoxy-1,3,5-tris-*O*-(trimethylsilyl) were identified in root methanol extracts; these components exhibit antioxidant activities (Mohamed and Khan 2013). Stem essential oils include 1,8-cineole (eucalyptol), *α*-caryophellene, *β*-pinene, and 9-epi-(*E*)-caryophellene as the major components (Alali *et al.* 2004).

Most of the studies related to the bioactivity of extracts from *S. persica* have focused on the extractives' effectiveness as a natural tool for dental cleaning and as a natural analgesic for toothache, as well as their effect on various aspects of oral health (Alali and Al-Lafi 2003; Balto *et al.* 2012; Halawany 2012; Chaurasia *et al*. 2013).

The antibacterial activities of extracts from several plants against bacterial potato pathogens have been assessed, and quite satisfactory results have been observed (Salem 2013; Ashmawy *et al.* 2014). The agricultural companies in the Mediterranean countries are focused on the commercial production of known aromatic herbs such as mint and basil (Edris *et al*. 2003) and neglecting the utilization of trees and shrubs, which may provide new sources of medical and agricultural applications (Bakkali *et al*. 2008; Abdel-Megeed *et al.* 2013; Salem *et al.* 2013, 2014a,b,c). So there is motivation to search for new and renewable sources for natural products that are useful against phytopathogenic bacteria and fungi (Salem *et al.* 2016a,b).

To date, there are no reports on the bioactivity of extracts from *S. persica* against the growth of pathogenic bacteria that attack plants. This study evaluated the antibacterial activity of extracts that analyzed by gas chromatography–mass spectrometry (GC/MS) from the leaves, branches, and root-wood of *S. persica* against the growth of some pathogenic bacteria.

EXPERIMENTAL

Plant Materials and Reagents

Leaves, branches, and root-wood of *Salvadora persica* were collected in May 2016 from the Jazan Region located on the southwestern part of the Kingdom of Saudi Arabia. The plant was identified by the Botany and Microbiology Department of the College of Science at King Saud University. The samples were delivered to the Faculty of Agriculture at Alexandria University by Dr. Hayssam M. Ali on June 2016. Extractions were performed at Alexandria University on the various *S. persica* components, and the antibacterial activity of extractives was assessed. The plant was authenticated with the voucher number Zidan0043. Methanol, dimethylsulfoxide (DMSO) and *n*-hexane solvents were bought from Sigma Aldrich (Cairo, Egypt).

Extraction

About 100 air-dried g of powdered leaf, branch, and root-wood were separately extracted by soaking in a mixture of methanol:*n*-hexane (1:1 *v/v*) for one week. The

extraction process was repeated three times in the week until exhaustion, where every filtration was done after two days. The combined extract from each plant part was concentrated using a rotary-evaporator at 45 °C. The concentrated extracts were stored for one week at 4 °C until further analysis. The extract weights from leaf, branch, and root-wood components were 6.24, 5.17, and 8.55 g, respectively. Each extract was prepared in the concentrations of (1000, 500, 250, 125, 64, and 32 µg/mL), by diluting the extract in 10% DMSO.

Antibacterial Activity Assay

The antibacterial activities of leaf, branch, and root-wood extracts from *S. persica* were evaluated using the disc diffusion method of Bauer *et al.* (1966) against the growth of selected phytopathogenic bacteria: *Pectobacterium carotovorum* subsp. *carotovorum* ippbc038, *Dickeya solani*, *Ralostonia solanacerum*, *Enterobacter cloacae*, and *Bacillus pumilus*. These bacterial strains have been associated with blackleg and soft rot disease of potatoes; also, these bacteria can completely destroy potato plantations, as well as cause brown rot in potatoes after post-harvest. The discs were impregnated with 20 µL of each of the concentrated extract (leaf, branch, and root-wood extracts). Mueller Hinton Agar (MHA) media in sterile Petri dishes were spread with a fresh 24-h-old bacterial suspension (1.0×10^5 CFU/mL) and sterile discs (Whatman filter paper no. 1) with 4 mm diameter and were stacked over the inoculated media surface. Three measurements of the inhibition zones around the discs were recorded in millimeters using a ruler.

The bacterial strains were supplied by the Department of Plant Pathology of the Faculty of Agriculture (El-Shatby) at Alexandria University (Alexandria, Egypt). Control discs with negative (DMSO) and positive (gentamicin 20 µg/disc) were performed, and all tests were performed in triplicate.

GC/MS Analyses of Extracts

The chemical compositions of the extracts were analyzed using 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 Centre of the Egyptian Atomic Energy Authority (Inshas, Cairo, Egypt). The column oven temperature was initially held at 120 °C and then increased by 5 °C·min^{-1} to 200 °C, which was held for 2 min, then increased to 280 °C (10 °C·min^{-1}). Temperatures of the injector and detector (MS transfer line) were kept at 250 °C. Helium, which was the carrier gas, was kept in constant flow rate of 1 mL·min^{-1}. 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. EI mass spectra were collected at 70 eV ionization voltages over the *m*/z range of 40 to 550 in full scan mode. The ion source and transfer line temperatures were set at 200 and 250 °C, respectively. 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 (Davies 1990).

Statistical Analysis

The values of the antibacterial activity are presented as mean of three replicates. Analysis of variance (ANOVA) was used to evaluate the significant difference among various treatments with the criterion of $p = 0.05$. The statistical analysis was performed

using SAS software version 8.2 (2001).

RESULTS AND DISCUSSION

Antibacterial Activity

As shown in Table 1, the root-wood extracts exhibited good bioactivity against the growth of *Ralostonia solanacerum* at 1000, 500, 250, 125, and 64 µg/mL levels with inhibition zone (IZ) values of 12.00, 11.33, 11.00, 11.00, and 11.00 mm, respectively. Furthermore, good bioactivity activity was observed with leaf extracts at 1000 µg/mL with an IZ value of 11.66 mm, and root-wood at 500 µg/mL (IZ value of 11.33 mm). The highest IZ values of the extracts was found against the growth of *Enterobacter cloacae*, with 11.00 mm for root-wood extracts at 1000, 500, and 250 µg/mL levels, followed by leaf extracts with 10.00 mm at the same concentration levels.

Table 1. Antibacterial Activity of Extracts from *S. persica* Leaf, Branch, and Root-Wood

Extract	Concentration (µg/ml)	*R. solanacerum* IZ (mm)	*E. cloacae* IZ (mm)	*B. pumilus* IZ (mm)	*P. carotovorum* IZ (mm)	*D. solani* IZ (mm)
BrSB	1000	8.00^{f}	8.00^{d}	11.33^{ab}	8.00^{ef}	10.33^{a}
	500	8.00^{f}	8.00^{d}	11.00^{bc}	7.00^{f}	9.66^{b}
	250	7.00^{g}	8.00^{d}	10.33^{cd}	7.00^{f}	9.00^{c}
	125	7.00^{g}	7.00^{e}	10.00^{d}	7.00^{f}	9.00^{c}
	64	7.00^{g}	7.00^{e}	9.00^{ef}	7.00^{f}	9.00^{c}
	32	7.00^{g}	7.00^{e}	8.66^{f}	7.00^{f}	9.00^{c}
LefSB	1000	11.66^{ab}	10.00^{b}	11.00^{bc}	11.00^{c}	10.00^{ab}
	500	10.33^{d}	10.00^{b}	10.33^{cd}	10.00^{cd}	9.66^{b}
	250	10.00^{d}	10.00^{b}	10.33^{cd}	10.00^{cd}	8.66^{c}
	125	10.00^{d}	9.00^{c}	10.00^{d}	9.66^{d}	9.00^{c}
	64	9.00^{e}	9.00^{c}	9.66^{de}	9.00^{de}	9.00^{c}
	32	9.00^{e}	8.00^{d}	9.00^{ef}	8.00^{ef}	9.00^{c}
RSB	1000	12.00^{a}	11.00^{a}	12.00^{a}	14.66^{a}	10.00^{ab}
	500	11.33^{bc}	11.00^{a}	11.00^{bc}	12.66^{b}	10.00^{ab}
	250	11.00^{c}	11.00^{a}	10.00^{d}	10.00^{cd}	9.00^{c}
	125	11.00^{c}	10.00^{b}	9.66^{de}	9.00^{de}	9.00^{c}
	64	11.00^{c}	9.00^{c}	9.00^{ef}	8.00^{ef}	9.00^{c}
	32	10.00^{d}	9.00^{c}	9.00^{ef}	8.00^{ef}	9.00^{c}
10% DMSO		na	na	na	na	na
Gentamicin*		34	22	23	18	30

Values are mean of three replicates. Means with the same letter within the same column are not significantly difference according to $LSD_{0.05}$. *Positive control; discs of 10 µg Gentamicin. BrSB: *S. persica* branch; LefSB: *S. persica* leaf; RSB: *S. persica* root-wood

For *Bacillus pumilus*, the most active extract was found from the root-wood at 1000 µg/mL with IZ value of 12.00 mm, which was followed by branch extracts with 11.33 mm at 1000 µg/mL. In addition, good activity (11.00 IZ value) was observed from extracts of branch (500 µg/mL), leaf (1000 µg/mL), and root-wood (500 µg/mL). Root-wood extracts showed good activity against *Pectobacterium carotovorum* with IZ value of 14.66 mm at 1000 µg/mL, followed by 12.66 mm at 500 µg/mL. Extracts of leaf showed some activity at 1000 µg/mL (11.00 mm), 500 µg/mL (10.00 mm), and 250

µg/mL (10.00 mm). Branch extracts showed activity against the growth of *Dickeya solani* at 1000 µg/mL with an IZ value of 10.33 mm, followed by leaf extracts at 1000 µg/mL (IZ 10.00 mm), and root-wood extracts at 1000 µg/mL (IZ 10.00 mm) and at 500 µg/mL (IZ 10.00 mm). Based on the these results, the root-wood extracts from *S. persica* had better antibacterial activity against the growth of the studied bacteria compared to leaf and branch extracts. Overall, the IZ values presented from the extracts are lower than those reported from the antibiotic used (Gentamicin).

All over the world, many trials have been done to control the diseases of potatoes without promising control. Some success has been reported with chemical control of brown rot (Murakoshi and Takahashi 1984), soil fumigants (Weingartner and Shumaker 1988), resistant varieties (Fock *et al.* 2001; Lopez and Biosca 2004), and antibiotics (Habashy *et al.* 1993). Additionally, chemical control (pesticides) with its residues has been reported to have hazardous effects in Europe and Egypt (Sylvander and Le Floc'h-Wadel 2000; Parrott and Kalibwani 2004).

Table 2. Identified Chemical Components of Methanol:*n*-Hexane Branch Extracts of *S. persica*

Retention Time (min)	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Area (%)
3.17	Decane	$C_{10}H_{22}$	142	3.17
4.09	1-(4,6-Bisbenzyloxyhexahydrocyclopenta [c]isoxazol-1-yl)ethanone	$C_{22}H_{25}NO_4$	367	1.25
5.20	1,6;3,4-Dianhydro-2-deoxy-*α*-D-ribohexo-pyranose	$C_6H_8O_3$	128	1.07
5.34	(5*α*)Pregnane-3,20*α*-diol,14*α*,18*α*-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)],diacetate	$C_{28}H_{43}NO_6$	489	1.29
10.41	3-Nitro-5-methyl-2-cyanomethylpyridine	$C_8H_7N_3O_2$	177	1.82
29.41	1-(4-Isopropylbenzylidenamino)-2-methyl-3-nitro-benzene	$C_{17}H_{18}N_2O_2$	282	1.03
30.60	Stigmasterol	$C_{28}H_{48}O$	412	3.17
31.65	*β*-Sitosterol	$C_{29}H_{50}O$	414	2.15
31.98	7-(6-Phenyl-n-hexyl)-3-phenylpyrazolo[1,5-a] pyrimidine	$C_{24}H_{25}N_3$	355	1.30
32.57	3-Desoxo-3,16-dihydroxy-12-desoxyphorbol-3,13,16,20-tetraacetate	$C_{28}H_{38}O_{10}$	534	1.47
32.72	(*Z*)-9-Octadecenoic acid (*Z*)-,9-octadecenyl ester	$C_{36}H_{68}O_2$	532	0.81
33.06	Dimethoxyglycerol docosyl ether	$C_{27}H_{56}O_5$	460	0.81
33.19	5,5''-Diethynyl-2,2':6',2''-terpyridine	$C_{19}H_{11}N_3$	281	0.98
33.34	10-Methoxy-6-methyl-methyl ester-(8*α*)-ergoline-8-carboxylic acid	$C_{18}H_{22}N_2O_3$	314	0.86
36.16	3-(2',6'-Dichlorobenzylidene)-1,3-dihydroindol-2-one	$C_{15}H_9Cl_2NO$	289	1.09
37.35	*N*-Benzylbenzamide	$C_{14}H_{13}NO$	211	71.08
37.91	Narceine	$C_{23}H_{27}NO_8$	445	1.08
38.17	17-Acetoxy-3*α*-methoxy-4,4-dimethyl-8,14-seco-3,19-epoxyandrostane-8,14-dione	$C_{24}H_{36}O_6$	420	1.07
38.38	9-Desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol	$C_{28}H_{40}O_{10}$	536	2.33
41.32	10-Hydroxy-5,7-dimethoxy-2,3-dimethyl-1,4-anthracenedione	$C_{18}H_{16}O_5$	312	1.21

Therefore, some studies have focused on using natural extracts for controlling potato disease. For example, Salem (2013) found that the bark extracts of *Delonix regia* and *Erythrina humeana* exhibited moderate antibacterial activity against the growth of potato soft rot bacteria *D. dianthicola*, *P. carotovorum* subsp. *wasabiae*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *atrosepticum*, and *D. chrysanthemi*. Additionally, the extracts from *Tecoma stans* leaves and branches also exhibited good activity compared with the extracts from *Callistemon viminalis* against the same bacteria strains. Salem *et al.* (2016a) found that the wood and bark extracts from *Picea abies* and *Larix decidua* showed moderate activity against the growth of *P. atrosepticum, P. carotovorum subsp. carotovorum* and *D. solani*. *Stenotrophomonas maltophilia*, isolated from the rhizosphere of eggplant cultivated in the Nile Delta of Egypt, was found to be potential biocontrol agent of *R. solanacerum* (Messiha *et al.* 2007).

Earlier studies of the antimicrobial effects of extracts from *S. persica* showed that the methanol extracts were less active than the aqueous extracts for the inhibition of *S. aureus*, *Streptococcus mutans*, *Streptococcus pyogenes*, *E. faecalis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and *Candida albicans* growth (Al-Bayati and Sulaiman 2008). The strong antimicrobial effects of extracts from *S. persica* against the growth of bacteria, fungi, and viruses have been attributed to volatile active compounds (Ali *et al.* 2002; Al-Mohaya *et al.* 2002; Hamza *et al.* 2006; Sofrata *et al.* 2008).

Chemical Constituents of Extracts

The methanol:*n*-hexane branch extracts of *S. persica* were analyzed by GC/MS, which identified 20 components (Table 2). The main chemical constituents in the extracts were: *N*-benzylbenzamide (71.08%), decane (3.17%), stigmasterol (3.17%), 9-desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol (2.33%), and *β*-sitosterol (2.15%).

GC/MS analysis of leaf extracts, which identified 23 components (Table 3), showed the presence of the following main components: 2,6-dimethyl-*N*-(2-methyl-*α*-phenylbenzyl)aniline (28.65%), spiculesporic acid (13.60%), homo-*γ*-linolenic acid (12.63%), methyl hexadecanoate (11.01%), and hexadecanoic acid (7.30%).

The GC/MS analysis of methanol:*n*-hexane extracts of the root-wood of *S. persica* (Table 4) identified six (6) main components: benzeneacetonitrile (71.47%), 4-aminocarbonyl-5-fluoro-1-*α*-D-ribofuranosyl-imidazole (10.99%), benzylisothiocyanate (5.05%), 2,2-dimethoxybutane (4.83%), *N*-benzylidenebenzylamine (4.36%), and 3-methyl-1-(phenylmethyl)azetidine (3.3%). Previously, most identified compounds were related to alkloidal constituents (*i.e.*, benzeneacetonitrile, and 2,6-dimethyl-*N*-(2-methyl-*α*-phenylbenzyl)aniline) (El-Mostehy *et al.* 1983; Malik *et al.* 1987; Bhandari 1990; Galletti *et al.* 1993; Darout *et al.* 2000; Alali and Al-Lafi 2003). In addition, carbohydrates, steroids, alkaloids, saponins, tannins, triterpenes, glycosides, mucilage, fats and oils have been reported from leaves and stems extracts of *S. oleoides* (Arora *et al.* 2014).

Phytol and *n*-hexadecanoic acid were the main components in leaf and stem extracts of *Salvadora oleoides* (Samejo *et al.* 2012). Butanediamide, *N*,*N*-bis(phenylmethyl)-2(*S*)-hydroxy-butanediamide, *N*-benzyl-2-phenylacetamide, *N*-benzylbenzamide, and benzylurea were isolated from the stems of *S. persica* (Khalil 2006). Fatty acids esters, such as oleic, linolic, and stearic acid, as well as some terpenoids, were investigated in the volatile compounds in *S. persica* crude extract (Abdelrahman *et al.* 2003). There were other volatile components, such as benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and *β*-caryophyllene (Alali and

Al-Lafi 2003). The aqueous extracts from roots and stems had antimicrobial components that were anionic, such as sulfate, chloride, thiocynate, and nitrate (Darout *et al.* 2000). Root extracts contain salvadourea (Ray *et al.* 1975) and benzylisothiocynate (Al-Bagieh 1990). Benzyl isothiocyanate was found to be highly active against Gram- Negative bacteria (Bader *et al.* 2002; Sofrata *et al.* 2011).

Table 3. Identified Chemical Components of Methanol:*n*-Hexane Leaf Extracts of *S. persica*

Retention Time (min)	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Area (%)
3.35	[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine	$C_{28}H_{34}N_2O$	414	2.75
3.70	1,2,3-Propanetriol	$C_3H_8O_3$	92	1.91
4.30	9-Hexyl-heptadecane	$C_{23}H_{48}$	324	0.36
4.77	Oxalic acid, isobutyltetradecyl ester	$C_{20}H_{38}O_4$	342	0.38
7.19	Benzeneacetonitrile	C_8H_7N	117	0.45
8.44	Dihydro-5-(1-hydroxyethyl)-2(3H)-furanone	$C_6H_{10}O_3$	130	0.58
9.44	Phenyl-propanedioic acid	$C_9H_8O_4$	180	0.77
13.96	1,4-Diazaspiro[4.4]non-3-en-1-yloxy-3-(dichloromethyl)-2,2-dimethyl-4-oxide	$C_{10}H_{15}Cl_2N_2O_2$	265	4.07
16.33	4,4,6-Trimethyltetrahydro-1,3-oxazine-2-thione	$C_7H_{13}NOS$	159	0.61
18.31	9-*cis*-Octadecenoic acid,(2-phenyl-1,3-dioxolan-4-yl)methyl ester	$C_{28}H_{44}O_4$	444	2.11
18.59	4-Fluorobenzoic acid tetradecyl ester	$C_{21}H_{33}FO_2$	336	1.08
24.68	Methyl-*cis*-9-hexadecenoate	$C_{17}H_{32}O_2$	268	0.47
24.99	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	11.01
25.22	2-Hydroxy-5,6-epoxy-15-methyl-pregan-20-one	$C_{22}H_{34}O_3$	346	0.40
25.48	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.30
26.43	1,4-Dimethyl-2-octadecylcyclohexane	$C_{26}H_{52}$	364	0.35
27.36	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	294	5.77
27.43	Spiculesporic acid	$C_{17}H_{28}O_6$	328	13.60
27.61	Phytol	$C_{20}H_{40}O$	296	0.98
27.72	Methyl 16-methylheptadecanoate	$C_{19}H_{38}O_2$	298	0.74
27.93	Homo-γ-linolenic acid	$C_{20}H_{34}O_2$	306	12.63
28.18	Flavone-4'-OH,5-OH,7-di-*O*-glucoside	$C_{27}H_{30}O_{15}$	594	0.65
37.30	2,6-Dimethyl-N-(2-methyl-α-phenylbenzyl)aniline	$C_{22}H_{23}N$	301	28.65

Table 4. Identified Chemical Components of Methanol:*n*-Hexane Root-Wood Extracts from *S. persica*

Retention Time (min)	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Area (%)
4.92	2,2-Dimethoxybutane	$C_6H_{14}O_2$	118	4.83
18.93	Benzeneacetonitrile	C_8H_7N	117	71.47
25.42	3-Methyl-1-(phenylmethyl)azetidine	$C_{11}H_{15}N$	161	3.3
27.19	Benzylisothiocyanate	C_8H_7NS	149	5.05
29.20	4-Aminocarbonyl-5-fluoro-1-α-D-ribofuranosyl-imidazole	$C_9H_{12}FN_3O_5$	261	10.99
36.84	*N*-Benzylidenebenzylamine	$C_{14}H_{13}N$	195	4.36

While the preliminary results from this study appear promising, further studies are recommended to justify the phytochemical application of *S. persica* extracts in the field as a natural pesticide for controlling phytopathogenic bacteria.

CONCLUSIONS

1. Leaf, branch, and root-wood extracts of *Salvadora persica* exhibited good antibacterial activity against the bacterial pathogens *Pectobacterium carotovorum subsp. carotovorum*, *Dickeya solani*, *Ralostonia solanacerum*, *Enterobacter cloacae*, and *Bacillus pumilus*, which attack potato plants.
2. The main chemical constituents in the branch extracts were *N*-benzylbenzamide, decane, stigmasterol, 9-desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol, and *β*-sitosterol. Additionally, the main constituents in the leaf extracts were 2,6-dimethyl-*N*-(2-methyl-*α*-phenylbenzyl)aniline, spiculesporic acid, homo-*γ*-linolenic acid, and methyl hexadecanoate. Likewise, the main constituents in the root-wood extracts were benzeneacetonitrile, 4-aminocarbonyl-5-fluoro-1-*α*-D-ribofuranosyl-imidazole, and benzylisothiocyanate.
3. The root-wood extracts from *S. persica* had better antibacterial activity against the growth of the studied potato bacterial pathogens compared to those reported from leaf and branch extracts, but all the inhibition zones were lower than the values reported from the antibiotic used (Gentamicin).

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