Potential impacts of Pinus halepensis *Miller trees as a source of phytochemical compounds: antibacterial activity of the cones essential oil and* n-*butanol extract*

Nader A. Ashmawy, Dunia A. Al Farraj, Mohamed Z. M. Salem, Mohamed S. Elshikh, Roua Al-Kufaidy, Mariyam K. Alshammari, et al.

Agroforestry Systems
An International Journal incorporating
Agroforestry Forum

ISSN 0167-4366
Volume 94
Number 4

Agroforest Syst (2020) 94:1403-1413
DOI 10.1007/s10457-018-0324-5

Springer

Agroforest Syst (2020) 94:1403–1413
https://doi.org/10.1007/s10457-018-0324-5

Potential impacts of *Pinus halepensis* Miller trees as a source of phytochemical compounds: antibacterial activity of the cones essential oil and *n*-butanol extract

Nader A. Ashmawy · Dunia A. Al Farraj · Mohamed Z. M. Salem · Mohamed S. Elshikh · Roua Al-Kufaidy · Mariyam K. Alshammari · Abdelfattah Z. M. Salem

Received: 1 September 2018 / Accepted: 15 November 2018 / Published online: 21 November 2018

Abstract The Aleppo pine (*Pinus halepensis* Miller) trees grown in the agricultural lands with high biodiversity, and considered as a potential source for chemical and therapeutic compounds. Essential oil (EO) and *n*-butanol fraction (But-fr) of Aleppo pine cones were evaluated against the growth of four plant bacterial pathogens (*Dickeya solani*, *Pectobacterium atrosepticum*, *Ralstonia solanacearum*, and *Agrobacterium tumefaciens*) and four human pathogenic bacteria (*Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, *Escherichia coli* ATCC 8739, and *Staphylococcus aureus* ATCC 6538). The diameter of the inhibition zone (IZ) and the minimum inhibitory concentrations (MICs) were measured. At 2000 µg/mL, But-fr showed the strongest activity against *D. solani*, *P. atrosepticum*, and *R. solanacearum* with inhibition zones (IZs) of 14.33 mm, 12.33 mm, and 15.33 mm, respectively. At 2000 µg/mL, EO showed the best activity against *A. tumefaciens* with an IZ value of 12.67 mm. Weak activity was observed by applying the EO and But-fr against *B. subtilis* and *S. lutea*, while good activity was recorded by But-fr against *E. coli* and *S. aureus* with IZs values of 13.67 mm and 11.33 mm, respectively, at 2000 µg/mL. Gas chromatography-mass spectrometry (GC/MS) analysis reported that the EO from cones contained mainly caryophyllene (15.17%), α-pinene (13.51%), and caryophyllene oxide (12.57%); But-fr contained 3,4-dimethyldihydrofuran-2,5-dione (36.25%), and 2-methylenecholestan-3-ol (18.12%). The phytochemical But-fr extract of Aleppo pine cones demonstrated moderate antibacterial effects against the studied bacteria.

Keywords *Pinus halepensis* · Cones · Pathogenic bacteria · Essential oil · *n*-butanol extract

N. A. Ashmawy
Department of Plant Pathology, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt

D. A. Al Farraj · M. S. Elshikh · R. Al-Kufaidy · M. K. Alshammari
Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

M. Z. M. Salem
Forestry and Wood Technology Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt

A. Z. M. Salem (✉)
Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
e-mail: asalem70@yahoo.com; salem@uaemex.mx

Introduction

Forests are consider to be the primary source of medicinal plants and valuable number forest products

such as fodder, fiber, food, cosmetics, gums, perfumes, resins, dyeing and plant protection (Zou and Sanford 1990; Joshi and Rawat 1997; FAO 2003). Medicinal and aromatic plants in agroforestry systems are playing an important role in discovering drugs for animal and human healthcare. Especially for people in villages and remote areas, which they are depend on medicinal and aromatic plants and their derivatives as primarily choose for treating human ailments (Nash et al. 1994; de Silva 1997; Prajapati et al. 2003; Rao et al. 2004). For example, the development of new drugs is strongly needed to cure infectious diseases caused by multi-resistant bacteria species (Rusenova and Parvanov 2009) and diseases caused by bacteria from horticultural plants such as soft, blackleg rot, and crown gall (De Cleene and De Ley 1976; Gardan et al. 2003).

In recent years, the search for antimicrobial activity in natural extracts has accelerated (Rios and Recio 2005). Natural products from higher and aromatic plants have been widely used as natural biocides against bacterial and fungal pathogens that cause diseases in humans and plants (Ashmawy et al. 2014; Chakotiya et al. 2017; El-Hefny et al. 2017a, b; Salem et al. 2018a).

The Aleppo pine is one of most important forest tree. All of the parts from this tree species have potential medicinal values, which could be useful for people in developing countries as a traditional product derived from forests. However, Aleppo pine is Mediterranean species growing in warmer calcareous areas like Morocco, Algeria, Libya, Turkey, Greece, and Italy (Tumen et al. 2010; Mohareb et al. 2017).

Phytochemicals such as essential oils (EOs), terpenes, turpentine, and phenolic compounds from Aleppo pine have valuable medicinal applications (Dob et al. 2005; Tumen et al. 2010; Abi-Ayad et al. 2010a, b; Nam et al. 2016; Mohareb et al. 2017). Traditionally, seeds of Aleppo pine are used for preparing a sweet pudding of group pine seeds throughout Arabic countries and was employed as an ingredient in ice creams Cheikh-Rouhou et al. 2006).

The needle EO is rich in β-caryophyllene, α-humulene, and aromadendren (Dob et al. 2005). The α-pinene, myrcene, (*E*)-β-caryophyllene, and caryophyllene oxide are the main compounds in the cone EO derived from the plant grown in Corsica (Nam et al. 2014). Moreover, α-pinene, myrcene, *p*-cymene, and (*Z*)-β-caryophyllene were designated as major components in the EO of needles (Djerrad et al. 2017). The Tunisian Aleppo pine have the (*Z*)-caryophyllene, β-myrcene, α-pinene, β-pinene, bicyclogermacrene, α-terpinolene, and α-humulene as the main chemical compounds (Hamrouni et al. 2015). Cones EO of the Turkish Aleppo pine contains α-pinene, β-caryophyllene, and caryophyllene oxide (Tumen et al. 2010). The main compounds in the EO from stems, cones, and needles of Aleppo pine are α-pinene, α-pinene, and (*Z*)-caryophyllene (Amri et al. 2013).

Various parts of the *Pinus* species (bark, needle, cone, and resin) have been used as antioxidants, antifungals, antibacterials, anti-inflammatories, and antiseptics (Sakagami et al. 1991; Dığrak et al. 1999; Salem et al. 2014a, b). The essential oils obtained from Aleppo pine cones have demonstrated the best effects on wound healing activity models, including anti-hyaluronidase activity and anti-inflammatory activity (Süntar et al. 2012).

The present work evaluated the antibacterial activity of essential oil and *n*-butanol extract from Aleppo pine cones grown in Egypt against the growth of some pathogenic bacteria. The phytochemical compositions of essential oil and *n*-butanol extract were analyzed using gas chromatography–mass spectrometry (GC/MS).

Materials and methods

Plant material

Cones of Aleppo pine were collected in September 2017 from Alexandria City, Egypt. The cones were air-dried under shaded conditions for one month and then ground into small pieces.

Isolation of essential oil and *n*-butanol fraction

The essential oil was extracted by hydrodistillation (Fig. 1). Small pieces of cones were first hydrodistillated by boiling 150 g of samples in 1500 mL distilled water for 3 h using a Clevenger extractor (Salem et al. 2013). After obtaining the essential oil, the material was filtrated to obtain the water suspended with dissolved extracts, and the residue or marc was discarded. The water extract was shaken with an equal volume of *n*-butanol using a funnel separator to

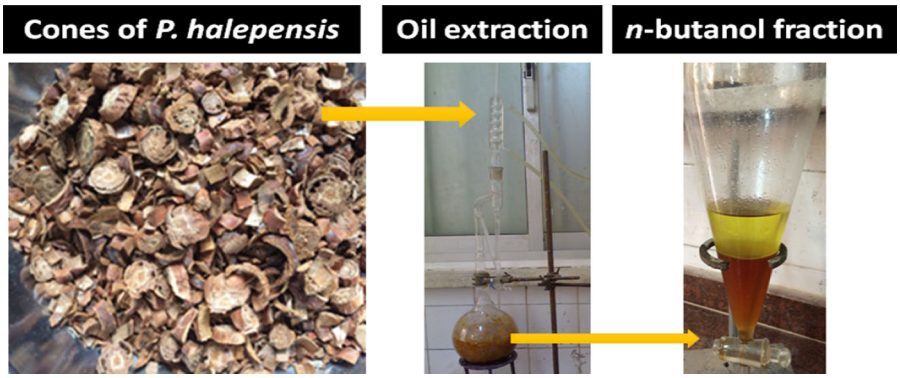

Fig. 1 Essential oil and *n*-butanol fraction isolated from Aleppo pine cones

produce two layers (Fig. 1). The *n*-butanol layer was concentrated under reduced pressure at 45 °C with a rotary evaporator, and the remainder (water layer) was discarded. The essential oil and *n*-butanol fraction extracted from cones of Aleppo pine were dissolved in 10% dimethyl sulfoxide to final concentrations of 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL, and 2000 µg/mL.

Antibacterial activity

Isolation and identification of phytopathogenic bacteria

Some plants showing natural infections, such as a potato tuber with soft rot or blackleg disease, and *Pyrus communis* with soft galls, were supplied from agricultural fields and stores. The diseased samples (rotted/galled tissues) were examined for the presence of bacterial colonies (Pérombelon and Van Der Wolf 2002; Moore et al. 2011; Toth et al. 2011; Czajkowski et al. 2015). The morphological and biochemical characteristics of the isolated plant bacteria were performed using standard methods (Cowan and Steel 1974; Klement et al. 1990; Staley et al. 2005).

Molecular identification

The extraction of DNA and molecular identification have been described in previous studies (De Cleene and De Ley 1976; Ashmawy et al. 2018; Behiry et al. 2018; Salem et al. 2018b). The following bacterial plant isolates were identified and used in the present work: *Dickeya solani*, *Pectobacterium atrosepticum*, *Ralstonia solanacearum*, and *Agrobacterium tumefaciens*.

Human bacterial pathogens

Four human pathogenic bacteria, namely *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, *Escherichia coli* ATCC 8739, and *Staphylococcus aureus* ATCC 6538, were used. Bacterial strains were supplied by the Laboratory of Bacteriology (Faculty of Agriculture, Alexandria University, Egypt).

Antibacterial activity

The media used to maintain bacteria and for the disc diffusion bioassay (NCCLS 1997) and minimum inhibitory concentrations assay (Eloff 1998) methods were nutrient agar (NA), Mueller–Hinton agar (MHA), and Mueller–Hinton Broth (MHB), respectively. Negative (10% dimethyl sulfoxide) and positive controls Tobramycin (10 µg/disc with human pathogenic bacteria) (Gentamicin, 20 µg/disc, with plant pathogenic bacteria) were used. All measurements were taken with three replicates. Minimum inhibitory concentrations (MICs) using serial dilutions of essential oil and *n*-butanol fraction ranged from 125 to 2000 µg/mL and were performed in 96-well microplates.

GC/MS analyses of essential oil and *n*-butanol fraction

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 was used to analysis the chemical composition of essential oil and *n*-butanol fraction from cones of Aleppo pine. The program temperatures of column oven, injector, and detector, as well as the properties of separation and identification, can be found in previously published work (El-Hefny et al. 2017b). The constituents were identified and performed on the basis of mass spectra library search (NIST and Wiley) (Adams 2007).

Statistical analysis

The resulting inhibition zones from the essential oil and *n*-butanol fraction against the growth of the studied bacterial pathogens were presented as mean ± standard deviation after analysis using the factorial experiment in the ANOVA test with two factors; the extracts and concentrations using the GLM procedure (SAS 2001).

Results and discussion

Antibacterial activity of essential oil and *n*-butanol fraction

The activity of essential oil (EO) and *n*-butanol fraction (But-fr) derived from cones of Aleppo pine at different concentrations against the plant bacterial pathogens are presented in Table 1. The highest inhibition zones (IZs) observed against *D. solani* were 14.33 mm and 11.67 mm as But-fr was applied at 2000 µg/mL and 1000 µg/mL. But-fr at 2000 µg/mL showed the highest activity against *P. atrosepticum* with IZ values of 12.33 mm at 2000 µg/mL. At 2000 µg/mL and 1000 µg/mL, the But-fr presented the highest activity against *R. solanacearum* with IZ values of 15.33 mm and 12.67 mm, respectively. The EO at 2000 µg/mL and 1000 µg/mL showed the best IZ values with 12.67 mm and 10.00 mm, respectively, against the growth of *A. tumefaciens*.

Table 2 shows that the EO and But-fr presented weak activity against *B. subtilis* and *S. lutea*. The But-fr showed good activity against the growth of *E. coli* with IZ values of 13.67 mm, 11.67 mm and 10.33 mm, at 2000 µg/mL, 1000 µg/mL, and 500 µg/mL, respectively. The EO at 2000 µg/mL and 1000 µg/mL showed IZ values of 12.33 mm, 11.67 mm, respectively, and But-fr at 2000 µg/mL showed an IZ value of IZ 11.33 mm, observed against the growth of *S. aureus*.

According to the data presented in Table 3, the MIC values were 2000 µg/mL (EO) with *B. subtilis*, 1000 µg/mL (EO) with *S. lutea*, 100 µg/mL (But-fr) with *E. coli*, < 125 µg/mL (EO and But-fr) with *S. aureus*, 100 µg/mL (But-fr) with *D. solani*, 225 µg/mL (But-fr) with *P. atrosepticum*, < 125 µg/mL (But-fr) with *R. solanacearum*, and 225 µg/mL (But-fr) with *A. tumefaciens*.

Phytochemical constituents of essential oil and *n*-butanol fraction

A total of 31 compounds were identified in the EO of Aleppo pine cones (Table 4), and the main constituents were caryophyllene (15.17%), α-pinene (13.51%), caryophyllene oxide (12.57%), methyl hexadecadienoate (10.81%), *d*-verbenol (7.74%), 2-pinen-4-one (6.02%), linoleic acid (4.48%), α-caryophyllene (3.79%), methyl 6-nonynoate (3.66%), *D,L*-isobornyl acetate (3.00%), epiglobulol (2.22%), and (*E*)-citral (2.19%).

In the But-fr, 12 compounds were identified with the following main constituents; 3,4-dimethyldihydrofuran-2,5-dione (36.25%), 2-methylenecholestan-3-ol (18.12%), (*Z*)-9-octadecenoic acid (13.45%), *trans*-2-undecenoic acid (12.55%), 1,7-octanediol,3,7-dimethyl- (6.23%), and ethyl iso-allocholate (4.09%) (Table 5).

The activity results reported from the IZs and MICs of EO and But-fr against the growth of plant and human bacterial pathogens ranged from weak to moderate inhibition.

The resistance of Gram-negative bacteria against EOs has been attributed to the presence of a hydrophilic outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Sakagami et al. 1991; Dığrak et al. 1999). The extract of Aleppo pine have shown a variable response against the growth of microorganisms. Less activity was observed with EO from Algeria against *E. coli* and *P. aeruginosa* (Abi-Ayad et al. 2010a).

Previously, it was found that EO from Aleppo pine is not active against the growth of *S. aureus* and *E. coli* (Fekih et al. 2014). On the other hand, other studies reported that the EO was affective against the growth

Table 1 Antibacterial activity of essential oil and extract from Aleppo pine cones against the growth of some plant pathogenic bacteria

Extract	Con. µg/mL	Diameter of inhibition zones[a] (mm ± standard deviation)											
		D. solani			*P. atrosepticum*			*R. solanacearum*			*A. tumefaciens*		
		IZ	− 95%	+95%	IZ	− 95%	+95%	IZ	− 95%	+95%	IZ	− 95%	+95%
Essential oil	125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.00	0.00	0.00
	500	6.00	6.00	6.00	0.00	0.00	0.00	0.00	0.00	0.00	8.33 ± 0.58	0.00	0.00
	1000	8.33 ± 0.58	6.90	9.77	8.33 ± 0.58	6.90	9.77	5.67 ± 0.58	4.23	7.10	10.00	6.00	6.00
	2000	9.67 ± 0.58	8.23	11.10	10.33 ± 0.58	8.90	11.77	7.33 ± 0.58	5.90	8.77	12.67 ± 0.58	5.23	8.10
n-butanol fraction	125	7.00	7.00	7.00	0.00	0.00	0.00	6.00	6.00	6.00	0.00	0.00	0.00
	250	8.00	8.00	8.00	6.00	6.00	6.00	7.67 ± 0.58	6.23	9.10	0.00	6.00	6.00
	500	10.00	10.00	10.00	8.00	8.00	8.00	9.33 ± 0.58	7.90	10.77	0.00	6.90	9.77
	1000	11.67 ± 0.58	10.23	13.10	9.67 ± 0.58	8.23	11.10	12.67 ± 1.15	9.80	15.54	6.00	10.00	10.00
	2000	14.33 ± 1.15	11.46	17.20	12.33 ± 0.58	10.90	13.77	15.33 ± 0.58	13.90	16.77	6.67 ± 0.58	11.23	14.10
Standard antibiotics and negative controls													
Tobramycin (10 µg)		15.33 ± 1.53			14.33 ± 1.53			12.67 ± 0.58			17.67 ± 0.58		
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

[b]Negative control, discs were loaded with 10% of dimethyl sulfoxide

Table 2 Antibacterial activity of essential oil and extract from Aleppo pine cones against the growth of some human pathogenic bacteria

Extract	Con. µg/mL	Diameter of inhibition zones[a] (mm ± standard deviation)											
		B. subtilis			*S. lutea*			*E. coli*			*Staph. aureus*		
		IZ	− 95%	+95%	IZ	− 95%	+95%	IZ	− 95%	+95%	IZ	− 95%	+95%
Essential oil	125	0.00	0.00	0.00	0.00	0.00	0.00	6.33 ± 0.58	4.90	7.77	0.00	0.00	0.00
	250	0.00	0.00	0.00	0.00	0.00	0.00	6.33 ± 0.58	4.90	7.77	6.33 ± 0.58	4.90	7.77
	500	0.00	0.00	0.00	0.00	0.00	0.00	7.00	7.00	7.00	7.67 ± 0.58	6.23	9.10
	1000	0.00	0.00	0.00	6.33 ± 0.58	4.90	7.77	7.00	7.00	7.00	11.67 ± 0.58	10.23	13.10
	2000	6.33 ± 0.58	4.90	7.77	7.67 ± 1.15	4.80	10.54	7.67 ± 0.58	6.23	9.10	12.33 ± 0.58	10.90	13.77
n-butanol fraction	125	0.00	0.00	0.00	0.00	0.00	0.00	7.67 ± 0.58	6.23	9.10	6.00	6.00	6.00
	250	0.00	0.00	0.00	0.00	0.00	0.00	8.67 ± 0.58	7.23	10.10	6.00	6.00	6.00
	500	0.00	0.00	0.00	0.00	0.00	0.00	10.33 ± 0.58	8.90	11.77	8.67 ± 0.58	7.23	10.10
	1000	0.00	0.00	0.00	0.00	0.00	0.00	11.67 ± 0.58	10.23	13.10	9.67 ± 0.58	8.23	11.10
	2000	0.00	0.00	0.00	6.00	6.00	6.00	13.67 ± 0.58	12.23	15.10	11.33 ± 0.58	9.90	12.77
Standard antibiotic and negative controls													
Gentamicin (10 µg)		15.33 ± 0.58			10.33 ± 0.58			10			14.33 ± 1.15		
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

[b]Negative control, discs were loaded with 10% of dimethyl sulfoxide

Table 3 Minimum inhibitory concentrations (MICs) of essential oil and *n*-butanol fr. for antibacterial activity

Extract	MIC value (μg/mL)							
	B. subtilis	*S. lutea*	*E. coli*	*S. aureus*	*D. solani*	*P. atrosepticum*	*R. solanacearum*	*A. tumefaciens*
Essential oil	2000	1000	125	< 125	500	1000	1000	225
n-butanol fraction	> 2000	>2000	100	< 125	100	225	< 125	1000

Table 4 Chemical constitutes of the essential oil from Aleppo pine cones

Compound name	RT (min.)	Relative peak area	Molecular formula	Molecular weight	Standard index	Reverse standard index
α-Pinene	8.12	13.51	$C_{10}H_{16}$	136	960	962
Camphene	8.94	0.54	$C_{10}H_{16}$	136	913	966
2,4-thujadiene	9.32	0.96	$C_{10}H_{14}$	134	852	901
β-Pinene	10.04	1.89	$C_{10}H_{16}$	136	881	914
Sabinene	10.80	0.44	$C_{10}H_{16}$	136	868	878
m-Cymene	12.12	0.70	$C_{10}H_{14}$	134	874	892
L-Borneol	14.99	0.63	$C_{10}H_{18}O$	154	699	853
α-Campholenal	16.56	1.39	$C_{10}H_{16}O$	152	877	917
d-Verbenol	17.16	7.74	$C_{10}H_{16}O$	152	879	881
3-Pinanone	18.11	0.43	$C_{10}H_{16}O$	152	780	849
Methyl 6-nonynoate	18.33	3.66	$C_{10}H_{16}O_2$	168	752	776
Myrtenol	19.01	0.76	$C_{10}H_{16}O$	152	823	849
3,6-Dimethyl-4,5,6,7-tetrahydro-1-benzofuran	19.27	0.46	$C_{10}H_{14}O$	150	773	818
Myrtenal	19.65	1.91	$C_{10}H_{14}O$	150	903	921
trans-carveol	19.87	0.43	$C_{10}H_{16}O$	152	826	852
2-Pinen-4-one	20.46	6.02	$C_{10}H_{14}O$	150	877	885
D,L-isobornyl acetate	21.06	3.00	$C_{12}H_{20}O_2$	196	900	908
(*E*)-citral	21.47	2.19	$C_{10}H_{16}O$	152	810	833
Cedrol	22.56	0.42	$C_{15}H_{26}O$	222	772	833
Methyl arachidonate	23.69	0.31	$C_{21}H_{34}O_2$	318	818	838
Caryophyllene	24.17	15.17	$C_{15}H_{24}$	204	936	950
α-Caryophyllene	25.13	3.79	$C_{15}H_{24}$	204	851	916
7,11-Hexadecadienal	25.94	0.48	$C_{16}H_{28}O$	236	743	774
α-Guaiene	26.37	1.96	$C_{15}H_{24}$	204	769	790
Epiglobulol	26.57	2.22	$C_{15}H_{26}O$	222	758	800
Caryophyllene oxide	28.23	12.57	$C_{15}H_{24}O$	220	920	938
linoleic acid	28.74	4.48	$C_{18}H_{32}O_2$	280	769	805
Methyl hexadecadienoate	29.18	10.81	$C_{17}H_{30}O_2$	266	826	833
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	30.31	0.21	$C_{16}H_{28}O_3$	268	794	820
[1,1′-Bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester	30.39	0.22	$C_{21}H_{38}O_2$	322	780	846
Oleic acid	30.47	0.68	$C_{18}H_{34}O_2$	282	833	841

Table 5 Profile of the chemical constitutes of the *n*-butanol fraction from Aleppo pine cones

Compound name	RT (min.)	Relative peak area	Molecular formula	Molecular weight	Standard index	Reverse standard index
3,4-Dimethyldihydrofuran-2,5-dione	3.43	36.25	$C_6H_8O_3$	128	839	920
Trans-2-undecenoic acid	5.88	12.55	$C_{11}H_{20}O_2$	184	649	689
1,7-Octanediol, 3,7-dimethyl-	7.72	6.23	$C_{10}H_{22}O_2$	174	715	732
3-Octanol	8.01	2.36	$C_8H_{18}O$	130	671	699
(*Z*)-9-Octadecenoic acid	10.22	13.45	$C_{18}H_{34}O_2$	282	756	756
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	22.27	2.54	$C_{16}H_{28}O_3$	268	708	713
2-Methylenecholestan-3-ol	23.73	18.12	$C_{28}H_{48}O$	400	741	749
Hexadecadienoic acid, methyl ester	26.56	0.86	$C_{17}H_{30}O_2$	266	768	781
1-Heptatriacotanol	27.09	1.53	$C_{37}H_{76}O$	536	804	815
Linoleic acid ethyl ester	27.54	0.83	$C_{20}H_{36}O_2$	308	746	750
Ethyl iso-allocholate	29.56	4.09	$C_{26}H_{44}O_5$	436	798	799
Docosanoic acid,8,9,13-trihydroxy-,methyl ester	30.86	1.17	$C_{23}H_{46}O_5$	402	784	784

of those bacteria (Hmamouchi et al. 2001; Abi-Ayad et al. 2010a; Saadou et al. 2013).

Caryophyllene oxide was found in 12.4 and 12.5% in some provenances of Aleppo pine grown in Algeria (Djerrad et al. 2017). Needle EO was found to be rich in *β*-caryophyllene (Dob et al. 2005). The main compound in the EO from cones of Aleppo pine *s* was α-pinene (Amri et al. 2013).

The plant grown in Turkey showed the presence of α-pinene, *β*-caryophyllene, and caryophyllene oxide in cones EO (Tumen et al. 2010). The α-pinene was the main compound in EO from cones (Amri et al. 2013). The main compounds, myrcene (27.9% and 42.1%), α-pinene (18.1% and 27.9%), and *β*-caryophyllene (16.4% and 14.3%) in needles and branches, respectively; α-pinene in cones (53.6%), were found in in the EO of Aleppo pine grown in Asciano (Pisa, Italy) (Macchioni et al. 2003).

Different environments, regions, soil conditions, and climates result in different chemical compositions of secondary metabolites in the same plant (Viljoen et al. 2005), i.e., the (*Z*)-caryophyllene compound from the EO of Aleppo pine has been shown in varied amounts in Italy, Morocco, Greece and Algeria (Panos et al. 1995; Dob et al. 2005). α-pinene, which was found in high amounts in Aleppo pine cone EO, has been shown to exhibit antifungal activity (Soković and Griensven 2006) and also displayed good antimicrobial activity (Matasyoh et al. 2007).

Throughout all civilization, the agroforestry tree Aleppo pine is considered one of the most popular plants, which contain pine compounds like turpentine, resins and EO that have economic traditional therapeutic practice in world (Baba Aissa 1991; Dob et al. 2007). This study reported the importance of using EO and *n*-butanol extract from Aleppo pine cones as biological agents against certain bacterial pathogens that cause diseases for both animal or human and plants (especially potato diseases).

Potato (*Solanum tuberosum* L.), is an important food crop worldwide. The origin of potato is the Andes, South America. It was introduced by the Spanish to Europe and from there, it was distributed to the other parts of the world, including Egypt. Potatoes are grown in two major zones, the temperate climate zone and the subtropical lowlands (Hijmans 2000a, b). This wide distribution of the potato crop makes it a staple food for highly populated areas worldwide. The year 2013 was declared by the FAO as the international year of the potato, (http://faostat.fao.org/default.aspx), which reflected the importance of the potato as a worldwide food crop.

In Egypt, many diseases were diagnosed on potato cultivated for either local consumption or exportation. Potato diseases cause a significant loss of the yield and

quality of potato (Kabeil et al. 2008). The diseases include some bacterial diseases such as brown rot (*R. solanacearum),* soft rot and blackleg diseases caused by *D. solani*, *P. atrosepticum*, were diagnosed on potato tubers (Kabeil et al. 2008; Ashmawy et al. 2015, 2018). The diseases listed above are limited factors for potato production in Egypt. So, the key factor for improvement potato production and exportation is development efficient methods for controlling these bacterial diseases. Crown gall induced by *A. tumefaciens* is a soil borne pathogen (Kerr and Brisbane 1983), which characterized by the formation of tumors at the wounded sites (Pionnat et al. 1999), the bacterium could be found as systematic infection in the xylem, or latent infection (Marti et al. 1998).

From the point of view of considering the agroforestry tree of Aleppo pine as a potential source of chemical compounds, terpenoids and phenolic compounds have reported to have a negative effect on root symbionts and site quality resulted from the interfering with mineralization, decomposition, and humification (Kainulainen and Holopainen 2002; Macchioni et al. 2003). Additionally, litters, leaf leachates, and root exudates have an allelopathic effects causing inhibition to seedling of various species and crop seeds (Fernandez et al. 2008; Alrababah et al. 2009; Navarro-Cano et al. 2009).

Conclusions

The current study demonstrated the potential uses of essential oil and *n*-butanol extract from the agroforestry tree of Aleppo pine (*P. halepensis*) cones against the growth of some pathogenic bacteria, four plant bacterial isolates, *D. solani*, *P. atrosepticum*, *R. solanacearum*, and *A. tumefaciens*, and four human pathogenic bacteria, *B. subtilis*, *S. lutea*, *E. coli*, and *S. aureus*. The *n*-butanol fraction had the highest activity, apart from the essential oil. Good activity was found when the *n*-butanol fraction was applied, especially against the plant pathogenic bacteria. The main compounds in the essential oil of Aleppo pine cones were caryophyllene, α-pinene, caryophyllene oxide, methyl hexadecadienoate, and *d*-verbenol (7.74%), and the main compounds in *n*-butanol fraction were 3,4-dimethyldihydrofuran-2,5-dione, 2-methylenecholestan-3-ol, (*Z*)-9-octadecenoic acid, and *trans*-2-undecenoic acid. The *n*-butanol fraction had good antibacterial activity, and more work should be carried out in vivo against the studied plant pathogens.

Acknowledgements The authors would like to thank the Deanship of Scientific Research at King Saud University for funding this work through research Group No. RG 1439-044.

References

Abi-Ayad M, Abi-Ayad FZ, Lazzouni HA, Rebiahi SA, Ziani-Cherif C, Bessiere JM (2010a) Chemical composition and antifungal activity of Aleppo pine essential oil. J Med Plant Res 5(22):5433–5436

Abi-Ayad M, Abi-Ayad FZ, Lazzouni HA, Rebiahi SA (2010b) Antibacterial activity of *Pinus halepensis* essential oil from Algeria (Tlemcen). J Nat Prod Plant Resour 1(1):33–36

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectroscopy, 4th edn. Allured Publishing, Carol Stream

Alrababah MA, Tadros MJ, Samarah NH, Ghosheh H (2009) Allelopathic effects of *Pinus halepensis* and *Quercus coccifera* on the germination of Mediterranean crop seeds. New Forest 38:261–272

Amri I, Hamrouni L, Hanana M, Gargouri S, Fezzani T, Jamoussi B (2013) Chemical composition, physico-chemical properties, antifungal and herbicidal activities of *Pinus halepensis* Miller essential oils. Biol Agric Hortic 29(2):91–106

Ashmawy NA, Behiry SI, Ali HM, Salem MZM (2014) Evaluation of *Tecoma stans* and *Callistemon viminalis* extracts against potato soft rot bacteria *in vitro*. J Pure App Microbiol 8(2):667–673

Ashmawy NA, Behiry SI, Younes HA, Khaled AE (2015) Development of polyclonal rabbit serum-based ELISA for detection of *Pectobacterium carotovorum* subsp. *carotovorum* and its specificity against other causing soft rot bacteria. Asian J Plant Pathol 9(3):135–141

Ashmawy NA, Salem MZM, El-Hefny M, Abd El-Kareem MSM, El-Shanhorey NA, Mohamed AA, Salem AZM (2018) Antibacterial activity of the bioactive molecules identified in three woody plants against some pathogenic bacteria. Microb Pathog 12:331–340

Baba Aissa F (1991) Medicinal plants in Algeria. Identification, description of active ingredient properties and traditional use of common plants in Algeria. Algiers, Bouchene and Ad. Diwan, p 181

Behiry SI, Ashmawy NA, Abdelkhalek AA, Younes HA, Khaled AE, Hafez EE (2018) Compatible and incompatible type interactions related to defense genes in potato elucidation by *Pectobacterium carotovorum*. J Plant Dis Prot 125(2):197–204

Chakotiya AS, Tanwar A, Narula A, Sharma RK (2017) *Zingiber officinale*: its antibacterial activity on *Pseudomonas aeruginosa* and mode of action evaluated by flow cytometry. Microb Pathog 107(6):254–260

Cheikh-Rouhou S, Hentati B, Besbes S, Blecker C, Deroanne C, Attia H (2006) Chemical composition and lipid fraction

characteristics of Alleppo pine (*Pinus halepensis* Mill.) seeds cultivated in Tunisia. Food Sci Technol Int 12(5):407–415

Cowan ST, Steel KJ (1974) Manual for the identification of medical bacteria, 2nd edn. Cambridge University Press, London

Czajkowski R, Pérombelon M, Jafra S, Lojkowska E, Potrykus M, van der Wolf J, Sledz W (2015) Detection, identification and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: a review. Ann Appl Biol 166(1):18–38

De Cleene M, De Ley J (1976) The host range of crown gall. Bot Rev 42(4):389–466

de Silva T (1997) In: Bodeker G, Bhat KKS, Burley J, Vantomme P (eds) Medicinal plants for forest conservation and healthcare. Non-wood forest products no. 11. FAO, Rome, pp 38–48

Dığrak M, İlçim A, Alma MH (1999) Antimicrobial activities of several parts of *Pinus brutia*, *Juniperus oxycedrus*, *Abies cilicia*, *Cedrus libani* and *Pinus nigra*. Phytother Res 13(7):584–587

Djerrad Z, Djouahri A, Kadik L (2017) Variability of *Pinus halepensis* Mill. Essential oils and their antioxidant activities depending on the stage of growth during vegetative cycle. Chem Biodivers 14(4):e1600340

Dob T, Berramdane T, Chelgoum C (2005) Chemical composition of essential oil of *Pinus halepensis* Miller growing in Algeria. C R Chimie 8(11–12):1939–1945

Dob T, Berramdane T, Chelghoum C (2007) Essential oil composition of *Pinus halepensis* Mill. from three different regions of Algeria. J Essent Oil Res 19:40–43

EL-Hefny M, Ali HM, Ashmawy NA, Salem MZM (2017a) Chemical composition and bioactivity of *Salvadora persica* extracts against some potato bacterial pathogens. BioRes 12(1):1835–1849

El-Hefny M, Ashmawy NA, Salem MZM, Salem AZM (2017b) Antibacterial activity of the phytochemicals-characterized extracts of *Callistemon viminalis*, *Eucalyptus camaldulensis* and *Conyza dioscoridis* against the growth of some phytopathogenic bacteria. Microb Pathog 113(12): 348–356

Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 64(8):711–713

FAO (2003) State of the World's Forests. FAO, Rome, p 153

Fekih N, Allali H, Merghache S, Chaïb F, Merghache D, El Amine M, Djabou N, Muselli A, Tabti B, Costa J (2014) Chemical composition and antibacterial activity of *Pinus halepensis* Miller growing in West Northern of Algeria. Asian Pac J Trop Dis 4(2):97–103

Fernandez C, Voiriot S, Mévy JP, Vila B, Ormeño E, Dupouyet S et al (2008) Regeneration failure of *Pinus halepensis* Mill.: the role of autotoxicity and some abiotic environmental parameters. For Ecol Manag 255(7):2928–2936

Gardan L, Gouy C, Christen R, Samson R (2003) Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum sp. nov., Pectobacterium betavasculorum sp. nov.* and *Pectobacterium wasabiae sp. nov*. Int J Syst Evol Microbiol 53(Pt 2):381–391

Hamrouni L, Hanan M, Amri I, Romane AE, Gargouri S, Jamoussi B (2015) Allelopathic effects of essential oils of *Pinus halepensis* Miller: chemical composition and study of their antifungal and herbicidal activities. Arch Phytopathol PFL 48(2):145–158

Hijmans RJ, Forbes GA, Walker TS (2000a) Estimating the global severity of potato late blight with a GIS-linked disease forecaster. Plant Pathol 49:697–705

Hijmans RJ, Garrett KA, Huamán Z, Zhang DP, Schreuder M, Bonierbale M (2000b) Assessing the geographic representativeness of genebank collections: the case of Bolivian wild potatoes. Conserv Biol 14:1755–1765

Hmamouchi M, Hamamouchi J, Zouhdi M, Bessiere JM (2001) Chemical and antimicrobial properties of essential oils of five Moroccan Pinaceae. J Essent Oil Res 13(4):298–302

Joshi DN, Rawat GS (1997) Need for conservation and propagation of alpine and sub-alpine medicinal plants of northwest Himalayas. Indian Forester 123:811–814

Kabeil SS, Lashin SM, El-Masry MH (2008) Potato brown rot disease in Egypt. J Agric Environ Sci 4(1):44–54

Kainulainen P, Holopainen JK (2002) Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. Soil Biol Biochem 34:37–42

Kerr A, Brisbane PG (1983) Agrobacterium. In: Fahy PC, Persley GJ (eds) Plant bacterial disease: a diagnostic guide. Academic Press, New York, pp 27–43

Klement K, Rudolph K, Sands DC (1990) Methods in phytobacteriology, vol 810. Akedemiai Kiado, Budapest

Macchioni F, Cioni PL, Flamini G, Morelli I, Maccioni S, Ansaldi M (2003) Chemical composition of essential oils from needles, branches and cones of *Pinus pinea*, *P. halepensis*, *P. pinaster* and *P. nigra* from central Italy. Flavour Fragr J 18(2):139–143

Marti R, Cubero J, Daza A, Piquer J, Salcedo CI, Morente C, Lòpez MM (1998) Evidence of migration and endophytic presence of *Agrobacterium tumefaciens* in rose plant. Eur J Plant Pathogen 50:1–12

Matasyoh JC, Kiplimo JJ, Karubiu NM, Hailstorks TP (2007) Chemical composition and antimicrobial activity of essential oil of *Tarchonanthus camphoratus*. Food Chem 101(3):1183–1187

Mohareb ASO, Kherallah IEA, Badawy MEI, Salem MZM, Hameda AY (2017) Chemical composition and activity of bark and leaf extracts of *pinus halepensis* and *Olea europaea* grown in AL-Jabel AL-Akhdar region, Libya against some plant phytopathogens. J Appl Biotechnol Bioeng 3(3):331–342

Moore LW, Kado CI, Bouzar H (2011) Agrobacterium. In: Schaad NW, Jones JB, Chun W (eds) Laboratory guide for identification of plant pathogenic bacteria. APS Press, St. Paul, pp 17–35

Nam AM, Casanova J, Tomi F, Bighelli A (2014) Composition and chemical variability of Corsican *Pinus halepensis* cone oil. Nat Prod Commun 9(9):1361–1364

Nam AM, Tomi F, Gibernau M et al (2016) Composition and chemical variability of the needle oil from *Pinus halepensis* growing in Corsica. Chem Biodivers 13(4):380–386

Nash RJ, Longland AC, Wormald MR (1994) The potential of trees as sources of new drugs and pesticides. In: Singh P, Pathak PS, Roy MM (eds) Agroforestry systems for

degraded lands, vol 2. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp 899–910

Navarro-Cano JA, Barbera GG, Ruiz-Navarro A, Castillo VM (2009) Pine plantation bands limit seedling recruitment of a perennial grass under semiarid conditions. J Arid Environ 73(1):120–126

NCCLS (1997) Performance standards for antimicrobial disk susceptibility tests: approved standard M2–A7. National Committee for Clinical Laboratory Standards, Wayne

Panos VR, Petrakis V, Ortiz A, Mazomenos BE (1995) Volatile constituents of needles of five *Pinus* species grown in Greece. Phytochemistry 39(2):357–361

Pérombelon MCM, Van Der Wolf JM (2002) Methods for the Detection and Quantification of *Erwinia carotovora* subsp. *Atroseptica* (*Pectobacterium carotovorum* subsp. *Atrosepticum*) on potatoes: a laboratory manual. Scottish Crop Research Institute, Invergowrie, p 393

Pionnat S, Keller H, Hericher D, Bettachini A, Dessaux Y, Nesme X, Poncet C (1999) Ti plasmids from *Agrobacterium* characterize rootstock clones that initiated a spread of crown gall disease in Mediterranean countries. Appl Environ Microb 65:4197–4206

Prajapati ND, Purohit SS, Sharma AK, Kumar T (2003) A handbook of medicinal plants. Agribios (India) 553:pp

Rao MR, Palada MC, Becker BN (2004) Medicinal and aromatic plants in agroforestry systems. Agroforest Syst 61:107–122

Rios JL, Recio MC (2005) Medicinal plants and antimicrobial activity. J Ethnopharmacol 100(1–2):80–84

Rusenova N, Parvanov P (2009) Antimicrobial activities of twelve essential oils against microorganisms of veterinary importance. Trakia J Sci 7(1):37–43

Saadou NC, Seridi R, Helamia F, Djahoudi A (2013) Chemical composition and antibacterial activities of Algerian *Pinus halepensis* Mill and *Pinus maritima* essential oils. Plant Med 79:114. https://doi.org/10.1055/s-0033-1352203

Sakagami H, Kawazoe N, Komatsu N, Simpson A, Nonoyama M, Konno K, Yoshida T, Kuroiwa Y, Tanuma S (1991) Antitumor, antiviral and immunopotentiating activities of pine cone extracts: potential medicinal efficacy of natural and synthetic lignin-related materials. Anticancer Res 11(2):881–888

Salem MZM, Ali HM, El-Shanhorey NA, Abdel-Megeed A (2013) Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: antibacterial and antioxidant activities, total phenolic and flavonoid contents. Asian Pac J Trop Med 6(10):785–791

Salem MZM, Ali HM, Basalah MO (2014a) Essential oils from wood, bark, and needles of *Pinus roxburghii* Sarg. from Alexandria, Egypt: antibacterial and antioxidant activities. BioRes 9(4):7454–7466

Salem MZM, Hatamleh AA, Ali HM (2014b) Efficacy of oily and resinous fractions of cone extracts from *Pinus roxburghii* Sarg. J Pure Appl Microbiol 8(5):3909–3913

Salem MZM, El-Hefny M, Ali HM, Elansary HO, Nasser RA, El-Settawy AAA, El Shanhorey N, Ashmawy NA, Salem AZM (2018a) Antibacterial activity of extracted bioactive molecules of *Schinus terebinthifolius* ripened fruits against some pathogenic bacteria. Microb Pathog 120:119–127

Salem MZM, Behiry SI, Salem AZM (2018b) Effectiveness of root-bark extract from *Salvadora persica* against the growth of certain molecularly identified pathogenic bacteria. Microb Pathog 117(4):320–326

SAS (2001) Users guide: statistics (release 8.02). SAS Institute, Cary

Soković M, Griensven LJLD (2006) Antimicrobial activity of essential oils and their components against the three major pathogens of cultivated button mushroom *Agaricus bisporus*. Eur J Plant Path 116(3):211–224

Staley JT, Boone DR, Garrity GM, Devos P, Fellow MG, Rainey FA, Schlifer KH, Brenner DJ, Castenholz RW, Holt JG, Krieg NR, Liston J, Moulder JW, Murray RGE, JrCF Niven, Pfenning N, Sneath PHA, Jully JG, Williams S (2005) Bergey's manual of systematic bacteriology: the proteobacteria, vol 2. Williams and Wilkins, Baltimore

Süntar I, Tumen I, Ustün O, Keles H, Akkol EK (2012) Appraisal on the wound healing and anti-inflammatory activities of the essential oils obtained from the cones and needles of *Pinus* species by in vivo and in vitro experimental models. J Ethnopharmacol 139(2):533–540

Toth IK, van der Wolf JM, Saddler G, Lojkowska E, Hélias V, Pirhonen M, Tsror L, Lahkim J, Elphinstone G (2011) *Dickeya* species: an emerging problem for potato production in Europe. Plant Pathol 60(3):385–399

Tumen I, Hafizoglu H, Kilic A, Dönmez IE, Sivrikaya H, Reunanen M (2010) Yields and constituents of essential oil from cones of *Pinaceae* spp. natively grown in Turkey. Molecules 15(8):5797–5806

Viljoen AM, Subramoney S, Vuuren SF, Başer KHC, Demirci B (2005) The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. J Ethnopharmacol 96(1–2):271–277

Zou X, Sanford RL (1990) Agroforestry systems in China: a survey and classification. Agroforest Syst 11:85–94