ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OILS, EXTRACTS AND SOME OF THEIR MAJOR COMPONENTS OF *Artemisia* spp. L. AGAINST SEED-BORNE PLANT PATHOGENIC BACTERIA

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ABSTRACT

This study was carried out to determine antibacterial activity of essential oils, extracts and major components of three *Artemisia* species (*A. absinthium, A. santonicum* and *A. spicigera)* against to some seed borne bacterial plant pathogens. According to our results, essential oils and some major components of *Artemisia* species have antibacterial activities at varying rates while extracts have shown no activity against any of the pathogens. Disc-diffusion method was used to test antimicrobial activity of the essential oils and extracts According to the results obtained, essential oil of *A. santonicum* has antibacterial effect against to 24 of 25 bacterial strains, essential oil of *A. absinthium* has antibacterial effect against 15 of 25 bacterial strains and essential oil of *A. spicigera* has antibacterial effect against only three of 25 bacterial strains. Additionally, constituents of the essential oils were analyzed by GC–MS method. Camphor, caryophyllene oxide, linalool, 1,8-cineole, terpinen-4-ol, borneol and α-terpineol were determined as predominant components. Minimum-maximum inhibition zones and MIC values of linalool were 8 mm (*C. violaceum* RK-231) - 45 mm (*X. campestris* pv. *vitians* RK-Xcvi), 50-110 mg/ml; terpinen-4-ol 8 mm (*B. pumilus* RK-106) - 43 mm (*X. campestris* pv. *vitians* RK-Xcvi) and MIC values 60-110 mg/ml; α-terpineol 8 mm (*P.cichorii* RK-166 and *X. axamopodis* pv. *vesicatoria* RK-399) – 10 mm (*P. huttiensis* RK-260 and *P. syringae* pv. *syringae* RK-204) and 60-70 mg/ml, respectively. But caryophyllene oxide, borneol, camphor and 1,8-cineole didn't show activity against any of the pathogens. In sum, our findings suggest that essential oils may be valuable as potential antibacterial agents against some plant pathogens.

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KEYWORDS: Antibacterial activity, essential oil, *Artemisia* spp., chemical composition

1. INTRODUCTION

Artemisia is a large, diverse genus of plants with between 200 and 400 species belonging to the daisy family Asteraceae. The genus is distributed worldwide, mainly across the temperate zones of the Northern Hemisphere, some species reaching the Arctic, but a few species can also be found on the Southern Hemisphere [1, 2]. *Artemisia* is represented by 23 species in the Turkish flora and among them; *A. absinthium*, *A. spicigera* and *A. santonicum* are found growing naturally in large areas of south-eastern Anatolia region of Turkey [3]. Members of this genus, have a characteristic scent or taste, have botanical and pharmaceutical interest, and are used in the liqueur-making industry. These herbs have been used worldwide in folk medicine since ancient times [4]. There are also several reports concerning the antimalarial, antioxidant, antibacterial, antidiabetic, and antifungal activities of different *Artemisia* species [5-9].

In recent years, crop loss is one of the major problems due to plant diseases caused by plant pathogen fungi, bacteria, viruses and insects. Microorganisms have also unfavorable effects on the quality, safety, and shelf life of foods. Nowadays, rapid and effective control of plant disease and microbial contamination in the crops is generally achieved using synthetic pesticides and sometimes, antibiotics. Control of plant bacterial diseases remains hard due to limited availability of commercial bactericides and prohibition of usage of the antibiotics in many countries. Thus, chemical control of bacterial diseases is largely dependent on the use of copper compounds. However, such control methods prevent bacterial multiplication but are not adequate against seed-borne inocula. However, these chemicals and antibiotics are associated with undesirable effects and some toxic residues in the products. Furthermore,

chemicals of this type evoke undesirable effects on the environment and leave residues toxic to mammalians [10, 11]. In addition, the risk of the development of resistance by microorganisms and the high cost-benefit ratio are other disadvantages of synthetic chemicals uses [12, 13]. In addition to microorganisms causing infectious diseases in humans may develop resistance to many antibiotics due to the indiscriminate use of commercial antibiotics [14, 15]. This problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, allergic reaction, and immunity suppression [16]. Therefore, there has been a growing interest in research concerning alternative pesticides and antimicrobial active compounds, including the plant extracts and essential oils that are relatively less damaging to the mammalian health and environment [11, 17, 18]. Hence, our interest focused on the effectiveness of the essential oils and extracts of *Artemisia* spp.

2. MATERIALS AND METHODS

2.1 Plant Pathogenic Bacterial Strains

Twenty-five bacterial strains used from culture collection of Assoc. Prof. Dr. Recep Kotan (Ataturk University, Agricultural Faculty, Turkey). Whole strains were tested formerly and they are highly virulence strains. All these strains had been determined as pathogens of different host plants [19-22] and were stored at -80°C in 15% glycerol and Luria Broth (LB) until use.

2.2 Plant Materials, Isolation of the Essential Oil and Extraction procedures

The aerial parts of *A. santonicum*, *A. spicigera* and *A. absinthium* were collected in Erzurum province of Turkey (eastern Turkey) in middle of July, at flowering stages and shaded for 7 days at room temperature. The voucher specimens have been deposited in the herbarium of Ataturk University, Erzurum (Turkey). The essential oils were isolated from the aerial parts of *A. absinthium*, *A. spicigera* and *A. santonicum* by hydrodistillation method using a Clevenger type apparatus. The yields were based on dried materials, shaded at room temperature for 7 days and determined over (w/w). The oils were dried over anhydrous Na_2SO_4 and stored under N_2 in a sealed vial until required.

The dried plant samples were powdered in a blender and then samples of 100 g extracted individually with n-hexane, chloroform, acetone and methanol at room temperature. After filtration, the organic solvents were evaporated under reduced pressure and temperature. For the methanol extract of the plant sample, the concentrated methanol extract was individually dissolved in distilled water (60°C) and then filtered. Thus, chlorophyll was removed from the solution. Then, this solution was lyophilized in a Labconco 117 freeze-dryer at 5 m-Hg and −50°C.

2.3 GC-MS Analyses

The oil composition was analyzed by gas chromatography–mass spectrometry (GC–MS). GC–MS analysis was performed using a Thermofinnigan Trace GC/Trace DSQ/A1300, (E.I. Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m×0.25 mm i.d., film thickness = 0.25 µm). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 to 150 °C at 3 °C /min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µl were injected manually in the splitless mode. The relative percentage of the oil constituents was expressed as percentages by peak area normalization. The identification of individual compounds of essential oils was based on comparison of their relative retention times with those of authentic samples on SGE-BPX5 capillary column, and by matching of their mass spectra of peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data [23, 24]. Authentic samples were purchased from Sigma, Fluka, Alfa or Aldrich. The relative percentages of major constituents of the oils are presented in Table 1. Pure major components, tested for antibacterial activity were purchased commercially from Fluka, Merck and Sigma.

2.4 Antibacterial Activity Assays

Antibacterial activity assays were carried out by disc diffusion method [25] with a minor modification using Tryptic Soy Agar (TSA, Merck, Germany) medium. The essential oil, extracts and pure compounds were prepared by dissolving using 10% dimethylsulfoxide (DMSO), and then were sterilized by filtration by 0.45 µm Millipore filters. Bacterial cultures were grown in Tryptic Soy Broth (TSB, Merck, Germany) and their suspension (100 µL) containing 1×10^8 CFU/ml of bacteria spread by a sterile swab on TSA medium. The discs (6 mm in diameter) were impregnated with 12.5 µl of the emulsions of the essential oils prepared in 10% DMSO distilled water, and with 10.0 mg/ml suspensions of the extracts and pure compounds prepared in 10% DMSO-distilled water. Then, they were put in the middle of the inoculated plates. The bacterial cultures were incubated at 27 ± 2 °C for 48 h, and then inhibition zones were measured in diameter (mm) around of the discs. Furthermore, bactericidal and bacteriostatic activities were also determined. The Tryptic Soy Agar samples taken from inhibited areas around of the discs were put into nutrient broth without essential oil, extracts and pure compounds incubated at 27 ± 2 °C for two days. After 48 h, whether there was no bacterial growth was observed in the broth culture, it was considered as bactericidal effect or not bactericidal. Oxacilin (1 µg/disc) and 10% DMSO–distilled water were used as positive and negative controls, respectively. All the tests were made in triplicate.

2.5 Determination of Minimal Inhibition Concentration (MIC).

Minimum inhibition concentrations (MICs) of the essential oils were tested by using a two-fold serial dilution

method [26]. Two fold serial dilutions of the liquid substances, essential oil was prepared by diluting 10% DMSO to achieve a decreasing concentration ranging from 500 µl/ml to 3.125 µl/ml. However, solutions of the solid substances, the extracts and fractions were prepared by diluting 10% DMSO at concentrations ranging from 50 to 110 mg/ml. Using 100 µl of suspension containing 1×10^8 CFU/ml was measured spectrophotometrically at 600 nm of bacteria spread on TSA plates. The blank discs (Oxoid) were impregnated with 12.5 µl of the solutions tested. Then, they were put in the middle of inoculated TSA plates. The bacterial cultures were incubated at 27 ± 2 ºC for 48 h. The lowest concentration of the essential oils, extracts and fractions showing a clear zone of inhibition were considered as the MIC. 10% DMSO was used as negative control. All the tests were carried out in triplicate.

3. RESULTS

3.1 The chemical composition of essential oils obtained by hydrodistillation method of aerial parts of plants

The hydrodistillation essential oil composition of Turkish three *Artemisia* species and the relative amounts of the components are shown in Table 1. This table has shown that the chemical composition of the three types of essential oil has differed from each other. In particular, the essential oil of *A. absinthium* is different than other types of essential oils. Essential oil of this type contains, chamazulene (17.77%), nuciferol butanoate (8.24%), nuciferol propionate (5.13%), caryophyllene oxide (4.28%) (*E*)-sabinene hydrate (2.87%), *cis*-sesquisabinene hydrate (2.67%), α-terpineol (2.38%) and geranyl isobutyrate (2.32%) as major components. Essential oils of *A. santonicum* and *A. spicigera* are more similar to each other in terms of chemical composition. These essential oils contain camphor (18.18 % and 34.85), 1,8-cineole (7.53% and 9.48%), cubenol (4.21% and 0.21%), borneol (4.02% and 5.10%), terpinen-4-ol (3.47% and 1.24%), α-terpineol (4.07% and 1.64%), α-selinene (2.38% and 0.49%) and bornyl acetate (2.17% and 1.00%) as major components, respectively.

3.2 Antibacterial test results of the main components in the essential oil

Table 2 represent the antibacterial activities of major components; namely linalool, terpinen-4-ol, α-terpineol, caryophyllene oxide, borneol, camphor and 1,8-cineole, obtained from essential oils linalool, terpinen-4-ol and α-terpineol has shown varying diameters of inhibition zones against pathogens, α-terpineol being the weakest. Caryophyllene oxide, borneol, camphor and 1,8-cineole didn't show activity against any of the pathogens.

Minimum-maximum inhibition zones and MIC values of linalool were 8 mm (*C. violaceum* RK-231) - 45 mm (*X. campestris* pv. *vitians* RK-Xcvi), 50-110 mg/ml; terpinen-4-ol 8 mm (*B. pumilus* RK-106) - 43 mm (*X. campestris* pv. *vitians* RK-Xcvi) and MIC values 60-110 mg/ml; α-terpin-

TABLE 1 - Chemical composition of the essential oils of test plants

RI[b]	Components	*A. absinthium* (%)	*A. santonicum* (%)	*A. spicigera* (%)	Identification methods
1042	**1,8-Cineole**	**1.48**	**7.53**	**9.48**	**GC, MS, RI**
1106	**Linalool**	**0.23**	**0.57**	**0.40**	**GC, MS, RI**
1153	**Camphor**	**1.41**	**18.18**	**34.85**	**GC, MS, RI**
1172	**Borneol**	**0.61**	**4.02**	**5.10**	**GC, MS, RI**
1178	**Terpinen-4-ol**	**1.80**	**3.47**	**1.24**	**GC, MS, RI**
1190	**α-Terpineol**	**2.38**	**4.07**	**1.64**	**GC, MS, RI**
1579	**Caryophyllene oxide**	**4.28**	**1.66**	**1.76**	**GC, MS, RI**
930	α-Thujene	-	0.10	-	GC, MS, RI
938	α-Pinene	-	0.59	-	GC, MS, RI
957	Camphene	-	1.00	0.10	GC, MS, RI
978	5-Methyl-3-hexen-2-one	-	-	0.27	MS
979	Sabinene	-	0.10	-	GC, MS, RI
983	β-Pinene	-	0.19	-	GC, MS, RI
988	3-Octanone	-	0.11	-	GC, MS, RI
994	β-Myrcene	0.19	0.54	0.10	GC, MS, RI
1012	α-Phellandrene	-	-	0.33	GC, MS, RI
1023	α-Terpinene	-	0.34	0.16	GC, MS, RI
1034	*p*-Cymene	0.61	0.39	0.49	GC, MS, RI
1037	Limonene	0.10	0.18	0.69	GC, MS, RI
1055	(*E*)-β-Ocimene	0.11	-	-	GC, MS, RI
1067	γ-Terpinene	-	0.45	-	GC, MS, RI
1071	Artemisia ketone	-	-	0.10	GC, MS, RI
1079	(*Z*)-Sabinene hydrate	-	0.98	-	GC, MS, RI
1080	*(E)*-Arbuscolene	-	-	0.31	MS,
1081	*cis*-Linalol oxide (furanoid)	0.41	-	-	GC,MS, RI
1084	Artemisia alcohol	-	-	0.10	MS, RI
1084	Camphenilone	-	-	0.35	MS, RI
1088	Fenchone	0.12	-	-	GC, MS, RI
1090	*trans*-Linalol oxide (furanoid)	0.27	-	-	MS, RI
1091	Terpinolene	-	0.13	-	GC, MS, RI
1095	*p*-Cymenene	0.10	-	-	MS, RI
1099	α-Pinene oxide	-	-	0.10	MS, RI
1103	Pentyl butyrate	-	0.13	-	MS, RI
1113	Isopentylisovalerate	-	0.26	-	MS, RI

1114	*cis*-Thujone	0.16	-	-	GC, MS, RI
1117	*(E)*-Sabinene hydrate	2.87	-	0.73	GC, MS, RI
1122	*trans*-Vertocitral C	-	1.15	-	MS, RI
1125	*trans*-Thujone	0.16	-	-	GC, MS, RI
1130	*cis-p*-Menth-2-en-1-ol	0.24	0.26	1.68	GC, MS, RI
1134	α-Campholenal	0.10	0.10	-	GC, MS, RI
1134	Terpinen-1-ol	-	-	0.30	MS, RI
1141	Nopinone	-	-	0.10	MS, RI
1143	Isocyclocitral	-	0.51	-	MS, RI
1145	*trans*-Pinocarveol	0.72	-	1.19	GC, MS, RI
1147	*trans-p*-Menth-2-en-1-ol	-	-	1.41	GC, MS, RI
1147	*trans*-Sabinol	-	0.44	-	MS, RI
1150	*trans*-Verbenol	0.29	0.27	-	GC, MS, RI
1158	Neo-3-Thujanol	0.10	-	-	MS, RI
1162	Sabinaketone	1.19	-	0.30	MS, RI
1163	Isoborneol	-	0.11	-	GC, MS, RI
1164	*cis*-Chrysanthenol	-	2.03	1.33	MS, RI
1170	*δ*-Terpineol	0.10	-	-	MS, RI
1185	Isomenthol	0.16	-	0.16	GC, MS, RI
1185	*p*-Cymen-8-ol	1.66	0.15	1.02	GC, MS, RI
1190	Myrtenol	eser	-	0.55	GC, MS, RI
1199	(*E*)-4-Decenal	-	-	0.10	MS, RI
1200	Verbanol	-	-	0.86	GC, MS, RI
1201	D-Verbenone	0.10	0.10	0.33	GC, MS, RI
1203	Isodihydrocarveol	-	0.12	-	GC, MS, RI
1210	*trans*-Pulegol	-	0.25	-	MS, RI
1211	*trans* -Carveol	0.12	-	0.23	GC, MS, RI
1212	*cis*-Sabinene hydrate acetate	-	-	0.26	MS, RI
1217	Nerol	0.49	0.10	-	GC, MS, RI
1218	Isobornyl formate	0.13	-	0.27	MS, RI
1242	Cuminaldehyde	0.89	-	0.49	GC, MS, RI
1243	*trans*-Chrysanthenyl acetate	0.10	0.76	0.12	MS, RI
1254	Piperitone	0.13	-	2.56	GC, MS, RI
1263	*cis*-Chrysanthenyl acetate	0.10	1.26	-	MS, RI
1264	Geranial	0.11	0.10	-	GC, MS, RI
1267	Nonanoic acid	0.10	-	0.13	GC, MS, RI
1273	Neoisopulegol acetate	-	-	0.16	MS, RI
1275	Isopulegol acetate	-	-	0.18	MS, RI
1278	Bornyl acetate	0.33	2.17	1.00	GC, MS, RI
1285	Lavandulyl acetate	-	0.73	-	MS, RI
1289	Thymol	0.31	0.27	0.58	GC, MS, RI
1287	(*E*)-Anethole	0.26	-	0.50	GC, MS, RI
1293	Phenyl 2-methylpropionate	-	0.10	-	MS, RI
1296	Carvacrol	0.48	0.12	0.32	GC, MS, RI
1322	2,4,6-trimethyl acetophenone	0.20	-	-	MS
1327	Methyl decanoate	-	-	0.11	MS, RI
1333	Isodihydrocarvyl acetate	-	-	0.11	MS, RI
1337	*trans*-Carveol acetate	0.36	0.14	0.22	GC, MS, RI
1346	α-Terpineol acetate	-	0.10	0.31	GC, MS, RI
1349	α-Longipinene	-	0.10	-	GC, MS, RI
1357	Eugenol	0.27	0.80	0.10	GC, MS, RI
1367	α-Yılangene	0.10	-	0.10	GC, MS, RI
1373	α-Copaene	0.18	0.10	0.40	GC, MS, RI
1377	Isobornyl propionate	-	0.12	-	MS
1383	β-Bourbonene	0.14	-	0.10	GC, MS, RI
1404	(Z)-Isoeugenol	0.10	0.44	0.57	MS, RI
1405	β-Isocomene	0.19	-	-	MS, RI
1406	Isoaryophyllene	0.20	-	0.26	GC, MS, RI
1412	Phenyl hexanal*	-	0.10	-	MS
1413	α-Cedrene	0.77	-	-	GC, MS, RI
1420	*cis-threo*-Davanofuran	-	-	0.10	MS, RI
1419	β-Caryophyllene	1.09	1.15	0.39	GC, MS, RI
1431	*p*-Cymen-7-ol acetate	-	-	0.14	MS, RI
1433	β-Gurjunene	0.10	-	-	GC, MS, RI
1442	Aromadendrene	0.10	-	-	GC, MS, RI
1453	(*Z*)-β-Farnesene	-	0.10	0.10	MS, RI
1460	α-Humulene	0.11	0.13	eser	GC, MS, RI
1463	α-Patchulene	-	0.10	-	MS, RI
1467	Linaly isovalerate	1.36	-	-	MS,RI
1470	Cyclamen aldehyde	0.10	-	-	MS, RI
1474	γ-Gurjunene	-	0.13	-	GC, MS, RI
1476	β-Chamigrene	0.10	0.43	0.20	MS, RI
1478	γ-Muurolene	0.16	0.11	-	GC, MS, RI
1484	α-Cyclogeraniol acetate	-	-	0.10	MS, RI
1486	Germacrene-D	-	1.30	-	GC, MS, RI
1486	*(E)*-β-Ionene	-	-	0.10	MS, RI
1488	β-Selinene	1.97	-	-	MS, RI
1493	Neryl isobutyrate	0.81	-	-	MS, RI
1499	α-Selinene	-	2.38	0.49	MS, RI
1500	Benzyl tiglate	-	-	0.10	MS, RI
1504	Neryl butyrate	0.53	-	-	MS, RI
1507	(*E,E*)-α-Farnesene	-	0.10	0.15	GC, MS, RI

1508	*(Z)*-α-Bisabolene	0.22	-	0.44	MS, RI
1513	γ-Cadinene	-	-	0.10	GC, MS, RI
1514	Cubebol	0.12	-	0.90	MS, RI
1515	Geranyl isobutyrate	2.32	0.10	-	MS, RI
1516	Dehydro-*ar*-γ-himachalene	0.10	-	-	MS, RI
1521	*(Z)*-Nerolidol	0.19	-	-	MS, RI
1522	*cis*-Calamenene	-	0.11	0.22	MS, RI
1523	Artedauglasia oxide-A	-	-	0.55	MS, RI
1539	*cis*-Sesquisabinenehydrate	2.67	-	-	MS, RI
1541	α-Calacorene	0.26	0.10	0.27	MS, RI
1546	Elemol	-	0.10	-	MS, RI
1551	Germacrene-B	-	0.10	-	MS, RI
1555	Geranyl butyrate	1.69	-	-	MS, RI
1555	(*E*)-Nerolidol	-	0.13	0.28	GC, MS, RI
1574	Spathulenol	1.75	1.31	3.70	GC, MS, RI
1585	2-Phenylethyl tiglate	-	-	0.33	MS, RI
1593	Globulol	-	-	0.20	MS, RI
1590	Gleenol	0.17	0.10	0.21	MS, RI
1593	Davanone	0.10	0.10	-	MS, RI
1595	Viridiflorol	0.30	-	0.26	MS, RI
1604	(*Z*)-Sesquilavandulol	0.38	0.44	0.83	MS, RI
1632	*(E)*-Sesquilavandulol	-	-	0.33	MS, RI
1633	β-Acorenol	0.24	-	-	MS, RI
1634	*epi*-α-Cadinol	0.40	-	-	MS, RI
1642	Cubenol	0.13	4.21	0.21	MS, RI
1650	(*Z*)-Methyl jasmonate	-	-	0.39	MS, RI
1651	Vulgarone-B	0.19	-	-	MS, RI
1654	Cedr-8(15)-en-9-α-ol	0.39	0.46	0.50	MS, RI
1656	Longipinocarvone	-	-	0.28	MS, RI
1658	α-Eudesmol	1.07	7.19	0.60	GC, MS, RI
1659	α-Cadinol	0.21	-	-	GC, MS, RI
1660	7-*epi*-α-Eudesmol	1.32	-	0.10	MS, RI
1682	Epi-α-Bisabolol	-	0.10	0.22	MS, RI
1690	α-Bisabolol	0.34	1.02	0.25	GC, MS, RI
1695	(*Z*)-α-*trans*-Bergamatol	-	0.10	0.11	MS, RI
1700	*n*-Heptadecane	-	-	0.10	GC, MS, RI
1731	(*E,Z*)-Farnesal	-	0.29	-	MS, RI
1736	14-Hydroxy-α-humulene	-	0.16	-	MS, RI
1745	Chamazulene	17.77	0.32	-	GC, MS, RI
1756	(*E,E*)-Farnesol	-	0.42	-	GC, MS, RI
1760	α-Bisabolol oxide A	-	0.25	-	MS, RI
1784	Benzyl benzoate	-	0.65	-	MS, RI
1790	(*Z*)-Lanceol	0.45	0.10	-	MS, RI
1794	Guaiazulene	0.89	-	-	GC, MS, RI
1796	*(E)*-α-Atlantone	0.15	-	-	MS, RI
1797	14-Hydroxy-α-muurolene	-	-	0.10	MS
1810	(*Z,E*)-Farnesyl acetate	-	2.53	-	GC, MS, RI
1838	(*E,E*)-Farnesyl acetate	-	0.10	-	GC, MS, RI
1844	(*Z,Z*)-Farnesyl acetone	1.20	0.10	0.12	MS, RI
1867	Diisobuthyl phthalate	1.33	0.26	0.15	MS, RI
1885	(Z)-Nuciferol acetate	0.29	-	-	MS, RI
1898	*(Z)*-Lanceol acetate	0.51	-	-	MS, RI
1902	Farnesyl propionate*	0.59	0.10	-	MS
1914	Nuciferol propionate*	5.13	2.05	-	MS
1916	Lanceol propionate*	0.90	1.28	-	MS
1900	*n*-Nonadecane	0.6	-	-	GC, MS, RI
1930	Cedrane-8,13-diol	0.20	-	-	MS
1930	Nuciferol butanoate*	8.24	0.53	-	MS
1932	Lanceol butanoate*	0.17	0.30	-	MS
1940	Cembrene	0.27	-	-	MS
1949	Lanceol pentanoate*	1.22	-	-	MS
1951	Phytol*	0.61	0.10	0.18	GC, MS, RI
1963	Ethyl hexadecanoate	0.36	-	-	MS
1964	Nuciferol hexanoate*	0.70	-	-	MS
Grouped components (%)					
Monoterpene hydrocarbons		1.11	3.62	1.38	
Oxygenated monoterpenes		23.58	50.34	70.11	
Sesquiterpene hydrocarbons		**6.42**	**7.26**	**2.93**	
Oxygenated sesquiterpenes		35.61	26.39	12.29	
Diterpenler		0.61	0.10	0.18	
Aromatic monoterpenes		22.83	3.82	3.91	
Others		3.30	0.96	1.47	
Total identified (%)		**93.46**	**92.49**	**92.27**	

tr, traces (less than 0.07%). [b]Retention index relative to n-alkanes on SGE-BPX5 capillary column; GC, identification was based on retention times of authentic compounds on SGE-BPX5capillary column; MS, identification was based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data [23]. RI, tentatively identified based on comparison of retention index of the com-pounds compared with published data [23].

TABLE 2 - Antibacterial activities of the major components.

Strains	Terpinen-4-ol		Linalool		α-Terpineol		1,8-Cineole		Borneol		Camphor		Caryophyllene oxide	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
A. piechaudii RK-155	11*	90.0	12*	90.0	–	–	–	–	–	–	–	–	–	–
B. pumilus RK-106	8*	90.0	10*	90.0	–	–	–	–	–	–	–	–	–	–
C. violaceum RK-231	11*	70.0	8*	110.0	–	–	–	–	–	–	–	–	–	–
C. michiganensis subsp. *michiganensis* RK-Cmm	9*	90.0	10*	110.0	–	–	–	–	–	–	–	–	–	–
E. intermedius RK-90	17*	80.0	14	90.0	–	–	–	–	–	–	–	–	–	–
E. amylovora RK-228	20*	70.0	13*	80.0	–	–	–	–	–	–	–	–	–	–
E. caratovora subsp. *atroceptica* RK-462	10*	90.0	18*	80.0	–	–	–	–	–	–	–	–	–	–
E. chrysanthemi RK-421	15*	80.0	14*	90.0	–	–	–	–	–	–	–	–	–	–
E. rhapontici RK-208	15*	70.0	13*	90.0	–	–	–	–	–	–	–	–	–	–
Flavobacter sp. RK-299	11*	90.0	29	90.0	–	–	–	–	–	–	–	–	–	–
P. agglomerans RK-84	12*	90.0	13*	80.0	–	–	–	–	–	–	–	–	–	–
P. aeruginosa RK-168	17*	70.0	–	–	–	–	–	–	–	–	–	–	–	–
P. cichorii RK-166	16	70.0	–	–	8	70.0	–	–	–	–	–	–	–	–
P. huttiensis RK-260	27*	60.0	25*	80.0	10	60.0	–	–	–	–	–	–	–	–
P. putida RK-249	9*	110.0	–	–	–	–	–	–	–	–	–	–	–	–
P. syringae pv. *syringae* RK-204	31*	60.0	26*	80.0	10	60.0	–	–	–	–	–	–	–	–
P. syringae pv. *tomato* RK-Pst1	26	80.0	34*	90.0	–	–	–	–	–	–	–	–	–	–
X. axamopodis pv. *malvacearum* RK-401	21*	70.0	33*	80.0	–	–	–	–	–	–	–	–	–	–
X. axamopodis pv. *vesicatoria* RK-399	25*	70.0	23*	60.0	8	70.0	–	–	–	–	–	–	–	–
X. campestris pv. *campestris* RK-Xcc	20	70.0	24	80.0	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *raphani* RK-Xcr	22*	70.0	14	80.0	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *vesicatoria* RK-Xcv1	36*	70.0	21*	80.0	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *vesicatoria* RK-Xcv 761	22*	70.0	21*	60.0	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *vitians* RK-Xcvi	43*	60.0	45*	50.0	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *zinia* RK-Xcz	9*	110.0	10*	110.0	–	–	–	–	–	–	–	–	–	–
X. malvacearum RK-397	22	70.0	21	80.0	–	–	–	–	–	–	–	–	–	–
X. pelargonii RK-406	21	70.0	33	80.0	–	–	–	–	–	–	–	–	–	–

IZ, inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; **MIC**, minimal inhibitory concentration as µLmL−1 for the essential oil and in mg mL−1 for the extracts. * Bactericidal effect was observed; – , not active.

eol 8 mm (*P.cichorii* RK-166 and *X. axamopodis* pv. *vesicatoria* RK-399) – 10 mm (*P. huttiensis* RK-260 and *P. syringae* pv. *syringae* RK-204) and 60-70 mg/ml, respectively.

Considering the test results of main components of essential oils, antibacterial effect is thought to arise from the substances such as linalool and terpinen-4-ol. It is believed that these substances are used for disinfection against pathogens, which is known as seed borne such as *X. campestris* pv. *campestris*, *X. campestris* pv. *vesicatoria* and *X. campestris* pv. *vitians*.

3.3 Antibacterial test results of the essential oils and extracts

In the present study, antibacterial activities of hydrodistillated essential oils, and the extracts isolated from the aerial parts of the plant species studied with *n*-hexane, CHCl3, acetone and methanol were tested against 25 plant pathogenic bacterial strains (Tables 3-5).

The results regarding the essential oil and extracts of *A. absinthium* have been shown in the Table 3. According to these results, essential oil of *A. absinthium* show inhibition zone against 15 of 25 bacterial strains in petri dishes while the extracts have no antibacterial effects. The highest inhibition zone with 15 mm was observed against the strains of *X. axonopodis* pv. *pelargonii* RK-Xa-pel. Also, the MIC value against this bacterial strain was 250 µl/ml.

The results regarding the essential oil and extracts of *A. santonicum* have been shown in the Table 4. According to these results, 24 of 25 bacterial strains were inhibited by essential oil of *A. santonicum* while the extracts have no antibacterial effects. Antibacterial activity defined against some strains is also important in terms of having a bactericidal effect. The highest inhibition zone with 29 mm was observed against the strains of *X. axonopodis* pv. *campestris* RK-Xa-cam. Also, the MIC value against this bacterial strain was 125 µl/ml.

The results regarding the essential oil and extracts of *A. spicigera* have been shown in the Table 5. According to these results, *A. spicigera* essential oil has antibacterial effect against to 3 of 25 bacterial strains while the extracts have no antibacterial effects. The highest inhibition zone with 16 mm was observed against the strains of *P. syringae* pv. *syringae* RK-204. Also, the MIC value against this bacterial strain was 500 µl/ml.

TABLE 3 - Antibacterial activities of the essential oil and extracts of *Artemisia absinthium*

Strains	Essential oil		Extracts								PC	NC
			Hexane		Chloroform		Aceton		Methanol		OX	10% DMS
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	IZ
A. piechaudii RK-155	–	–	–	–	–	–	–	–	–	–	–	–
B. pumilus RK-106	–	–	–	–	–	–	–	–	–	–	11	–
C. violaceum RK-231	–	–	–	–	–	–	–	–	–	–	–	–
C. michiganensis subsp. *michiganensis* Cmm	–	–	–	–	–	–	–	–	–	–	11	–
E. intermedius RK-90	–	–	–	–	–	–	–	–	–	–	–	–
E. amylovora RK-228	–	–	–	–	–	–	–	–	–	–	–	–
E. caratovora subsp. *atroceptica* RK-462	7	500	–	–	–	–	–	–	–	–	–	–
E. chrysanthemi RK-421	9	500	–	–	–	–	–	–	–	–	–	–
E. rhapontici RK-208	–	–	–	–	–	–	–	–	–	–	–	–
Flavobacter sp. RK-299	11	500	–	–	–	–	–	–	–	–	–	–
P. agglomerans RK-84	–	–	–	–	–	–	–	–	–	–	–	–
P. aeruginosa RK-168	7	500	–	–	–	–	–	–	–	–	–	–
P. cichorii RK-166	–	250	–	–	–	–	–	–	–	–	7	–
P. huttiensis RK-260	7	250	–	–	–	–	–	–	–	–	–	–
P. putida RK-249	–	–	–	–	–	–	–	–	–	–	7	–
P. syringae pv. *syringae* RK-204	11	250	–	–	–	–	–	–	–	–	–	–
P. syringae pv. *tomato* RK-Ps-tom	7	500	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	13	500	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *vesicatoria* Xcv110c	9	500	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *campestris* RK-Xa-cam	9	500	–	–	–	–	–	–	–	–	7	–
X. campestris pv. *raphani* RK-Xc-rap	8	500	–	–	–	–	–	–	–	–	7	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	7	250	–	–	–	–	–	–	–	–	8	–
X. axonopodis pv. *vitians* Xa-vit	7	500	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *zinniae* Xc-zin	10	250	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	**15**	**250**	–	–	–	–	–	–	–	–	7	–

IZ, inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; **MIC**, minimal inhibitory concentration as µLmL−1 for the essential oil and in mg mL−1 for the extracts; **PC,** positive control (OX: Oxacilin), **NC,** Negative control, **–** , not active, .

TABLE 4 - Antibacterial activities of the essential oil and extracts of *Artemisia santonicum*

Strains	Essential oil		Extracts								PC	NC
			Hexane		Chloroform		Aceton		Methanol		OX	10% DMS
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	IZ
A. piechaudii RK-155	10	250	–	–	–	–	–	–	–	–	–	–
B. pumilus RK-106	11	250	–	–	–	–	–	–	–	–	11	–
C. violaceum RK-231	9	125	–	–	–	–	–	–	–	–	–	–
C. michiganensis subsp. *michiganensis* Cmm	–	–	–	–	–	–	–	–	–	–	11	–
E. intermedius RK-90	9	250	–	–	–	–	–	–	–	–	–	–
E. amylovora RK-228	14*	250	–	–	–	–	–	–	–	–	–	–
E. caratovora subsp. *atroceptica* RK-462	15*	250	–	–	–	–	–	–	–	–	–	–
E. chrysanthemi RK-421	9	125	–	–	–	–	–	–	–	–	–	–
E. rhapontici RK-208	16	125	–	–	–	–	–	–	–	–	–	–
Flavobacter sp. RK-299	20*	125	–	–	–	–	–	–	–	–	–	–
P. agglomerans RK-84	10	500	–	–	–	–	–	–	–	–	–	–
P. aeruginosa RK-168	7	–	–	–	–	–	–	–	–	–	–	–
P. cichorii RK-166	7	–	–	–	–	–	–	–	–	–	7	–
P. huttiensis RK-260	12	125	–	–	–	–	–	–	–	–	–	–
P. putida RK-249	11	250	–	–	–	–	–	–	–	–	7	–
P. syringae pv. *syringae* RK-204	28	125	–	–	–	–	–	–	–	–	–	–
P. syringae pv. *tomato* RK-Ps-tom	11	250	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	27*	125	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *vesicatoria* Xcv110c	15*	125	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *campestris* RK-Xa-cam	29*	125	–	–	–	–	–	–	–	–	7	–
X. campestris pv. *raphani* RK-Xc-rap	14	125	–	–	–	–	–	–	–	–	7	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	17*	125	–	–	–	–	–	–	–	–	8	–
X. axonopodis pv. *vitians* Xa-vit	15	125	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *zinniae* Xc-zin	11	125	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	15	125	–	–	–	–	–	–	–	–	7	–

IZ, inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; **MIC**, minimal inhibitory concentration as µLmL−1 for the essential oil and in mg mL−1 for the extracts. * Bactericidal effect was observed; **PC,** positive control (OX: Oxacilin), **NC,** Negative control, – , not active.

TABLE 5 - Antibacterial activities of the essential oil and extracts of *Artemisia spicigera*

Strains	Essential oil		Extracts								PC	NC
			Hexane		Chloroform		Aceton		Methanol		OX	10% DMS
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	IZ
A. piechaudii RK-155	–	–	–	–	–	–	–	–	–	–	–	–
B. pumilus RK-106	–	–	–	–	–	–	–	–	–	–	11	–
C. violaceum RK-231	–	–	–	–	–	–	–	–	–	–	–	–
C. michiganensis subsp. *michiganensis* Cmm	–	–	–	–	–	–	–	–	–	–	11	–
E. intermedius RK-90	–	–	–	–	–	–	–	–	–	–	–	–
E. amylovora RK-228	12	500	–	–	–	–	–	–	–	–	–	–
E. caratovora subsp. *atroceptica* RK-462	–	–	–	–	–	–	–	–	–	–	–	–
E. chrysanthemi RK-421	–	–	–	–	–	–	–	–	–	–	–	–
E. rhapontici RK-208	–	–	–	–	–	–	–	–	–	–	–	–
Flavobacter sp. RK-299	–	–	–	–	–	–	–	–	–	–	–	–
P. agglomerans RK-84	–	–	–	–	–	–	–	–	–	–	–	–
P. aeruginosa RK-168	–	–	–	–	–	–	–	–	–	–	–	–
P. cichorii RK-166	–	–	–	–	–	–	–	–	–	–	7	–
P. huttiensis RK-260	–	–	–	–	–	–	–	–	–	–	–	–
P. putida RK-249	–	–	–	–	–	–	–	–	–	–	7	–
P. syringae pv. *syringae* RK-204	16	500	–	–	–	–	–	–	–	–	–	–
P. syringae pv. *tomato* RK-Ps-tom	–	–	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	–	–	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *vesicatoria* Xcv110c	–	–	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *campestris* RK-Xa-cam	–	–	–	–	–	–	–	–	–	–	7	–
X. campestris pv. *raphani* RK-Xc-rap	–	–	–	–	–	–	–	–	–	–	7	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	10	500	–	–	–	–	–	–	–	–	8	–
X. axonopodis pv. *vitians* Xa-vit	–	–	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *zinniae* Xc-zin	–	–	–	–	–	–	–	–	–	–	–	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	–	–	–	–	–	–	–	–	–	–	7	–

IZ, inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; **MIC**, minimal inhibitory concentration as µLmL−1 for the essential oil and in mg mL−1 for the extracts; **PC,** positive control (OX: Oxacilin), **NC,** Negative control, **–** , not active.

4. DISCUSSION AND CONCLUSIONS

Our results showed that essential oils (12.5 µL disc^{-1}) of *A. absinthium, A. santonicum* and *A. spicigera* exhibited a broad spectrum of potent antibacterial activity against some tested bacterial strains producing 7–29 mm inhibition zones depending on the bacterial strains tested. However the extracts have no antibacterial effects. The results presented in Tables 3, 4 and 5 show that the MIC values of the oils vary with the bacterial strains tested, ranging from 125.0 to 500.0 µLmL^{-1}. Furthermore, *A. santonicum* oil had bactericidal activity against 7 bacterial strains tested (Table 2), whereas *A. absinthium* and *A. spicigera* oils didn't show any bactericidal activity against all of the pathogen strains (Table 1,3). Numerous reports indicated that a high percentage of essential oil having antimicrobial activity [27-32]. The oil of *A. absinthium* showed the weak antibacterial activity at a broader spectrum. However, the antibacterial effect of *A. spicigera* was found to be low as compared to the essential oil of *A. santonicum* and *A. absinthium* with regard to low inhibition zones and high MIC values. However, *A. santonicum* were active against most of the bacterial strains. Previously, we have reported that the essential oils of *A. santonicum* species have chemical compositions and major components such as terpinen-4-ol, camphor, 1,8- cineole and borneol [8]. The wide antibacterial spectra of *A. santonicum* oil may also be attributed to their relatively high content of oxygenated monoterpenes [8]. Recently, oxygenated monoterpenes such as camphor, 1,8- cineole, terpinen-4-ol, and borneol, which were detected in the oils of *A. santonicum* was reported to exhibit antibacterial activity [33-35]. These reports are compatible with our results in the present study.

Although there are numerous reports on the analyses of essential oils from *Artemisia* species in the literature, some *Artemisia* oils were tested against only a limited number of bacteria [6, 8]. On the other hand, using essential oils of the *Artemisia* species against seed-borne bacterial pathogens is not enough work available. Therefore, this study performed has a great importance.

In conclusion, the development of natural antimicrobials will help to decrease the negative effects (residues, resistance, and environmental pollution) of synthetic drugs. In this respect, natural antimicrobials may be also effective, selective, biodegradable, and less toxic to environment. In addition, the risk of the development of resistance by microorganisms and the high cost-benefit ratio are other disadvantages of synthetic chemicals uses [13]. In addition to microorganisms causing infectious diseases in humans may develop resistance to many antibiotics due to the indiscriminate use of commercial antibiotics [14]. This problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, allergic reaction, and immunity suppression [36]. Thus, this study matters to highlight the successful usage an environment-friendly, natural, risk free for health of humans and other livings product against some seed-borne pathogens in substitution for the chemical pesticides that are intensely used and harmful for environment, natural balance and human health. In view of

the present results, it is concluded that these essential oils can be used as antimicrobial agents for control of seed-borne bacterial plant pathogens. However, the safety and toxicity of these compounds will need to be addressed.

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