Research Article

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Antibacterial activities of essential oils and extracts of Turkish *Achillea, Satureja* and *Thymus* species against plant pathogenic bacteria

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Abstract

BACKGROUND: The aims of this study were to examine the chemical composition of the essential oils and hexane extracts of the aerial parts of *Satureja spicigera* (C. Koch) Boiss., *Thymus fallax* Fisch. & CA Mey, *Achillea biebersteinii* Afan, and *Achillea millefolium* L. by GC and GC–MS, and to test antibacterial efficacy of essential oils and *n*-hexane, chloroform, acetone and methanol extracts as an antibacterial and seed disinfectant against 25 agricultural plant pathogens.

RESULTS: Thymol, carvacrol, *p*-cymene, thymol methyl ether and γ-terpinene were the main constituents of *S. spicigera* and *T. fallax* oils and hexane extracts. The main components of the oil of *Achillea millefolium* were 1,8-cineole, δ-cadinol and caryophyllene oxide, whereas the hexane extract of this species contained mainly *n*-hexacosane, *n*-tricosane and *n*-heneicosane. The oils and hexane extracts of *S. spicigera* and *T. fallax* exhibited potent antibacterial activity over a broad spectrum against 25 phytopathogenic bacterial strains. Carvacrol and thymol, the major constituents of *S. spicigera* and *T. fallax* oils, also showed potent antibacterial effect against the bacteria tested. The oils of *Achillea* species showed weak antibacterial activity. Our results also revealed that the essential oil of *S. spicigera*, thymol and carvacrol could be used as potential disinfection agents against seed-borne bacteria.

CONCLUSION: Our results demonstrate that *S. spicigera, T. fallax* oils, carvacrol and thymol could become potentials for controlling certain important agricultural plant pathogenic bacteria and seed disinfectant.
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Keywords: *Achillea*; antibacterial activity; camphor; carvacrol; phytopathogens; *Satureja*; *Thymus*; thymol; 1,8-cineole; seed disinfection

INTRODUCTION

Crop loss is one of the major problems due to plant diseases caused by plant pathogenic bacteria, fungi, viruses and insects. Furthermore, seed-borne bacterial and fungal diseases can be spread with seeds.[1] Therefore, the use of healthy seeds is the most important factor for controlling the seed borne diseases. Today, rapid and effective control of plant disease and microbial contamination in crop cultivation is generally achieved by use of synthetic pesticides and antibiotics. However, such control methods of the plant diseases prevent bacterial multiplication but are not always adequate control methods for seed-borne inoculums. These chemicals are also associated with undesirable effect on the environment due to their slow biodegradation in the environment and some toxic residues in the products for mammalian health.[2] This issue is still in the centre of debate although the use of antibiotics is forbidden in many European Union countries. Only the United States and a few other countries allow the use of oxytetracycline and streptomycin for the control of bacterial diseases on important crops.[3] In addition, the risk of developing resistance by microorganisms and the high cost–benefit ratio are other disadvantages of synthetic pesticide uses.[4] Therefore, there has been a growing interest in research concerning the alternative pesticides and antimicrobial active compounds, including the plant extracts and essential oils.[2,5–11]

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Table 1. Yields (%) of essential oils and extracts isolated from the plant species

Plant sample	Essential oil (g 100 g^{-1} plant sample)	Extracts (g 100 g^{-1} plant sample)			
		Hexane	Chloroform	Acetone	Methanol
Achillea biebersteinii	0.66	2.06	5.98	4.48	6.62
Achillea millefolium	0.90	2.08	5.44	2.63	5.52
Satureja spicigera	1.56	2.74	4.76	4.94	18.96
Thymus fallax	1.95	3.34	4.64	2.6	6.72

Thymus fallax and *Satureja spicigera*, belonging to Lamiacaeae family, are well known aromatic and medicinal plants and are distributed in northern Anatolia.[12,13] In Anatolia, *Thymus* (thyme) and *Satureja* (savory) species are frequently used as tea or additives in commercial spice mixtures of many foods to offer aroma and flavour.[13] The essential oils isolated from *Satureja spicigera* and *Thymus fallax* from Turkey and different locations of the world have been previously reported.[14–19] There are numerous reports on the chemical composition and antibacterial activities of the essential oils from various *Thymus* and *Satureja* species against human and food pathogenic bacteria.[6,7,9,20,21] However, there are a few reports on the antibacterial effects of the essential oils of *Thymus* and *Satureja* species against a few phytopathogenic bacteria.[5,8,11,22,23]

The genus *Achillea* is one of the most important genera of the Asteraceae family and there are about 42 species of this genus in Turkish flora and about 20 of them are endemic.[13,24] The species of *Achillea* genus are known in Anatolia as 'Civan perçemi', 'Pireotu' and 'Yılan çiçeği'.[13] The chemical compositions of the essential oils of *A. millefolium* and *A. biebersteinii,* and their antibacterial activities against various bacteria have been previously reported.[25–29] However, there is no report concerning the chemical analyses of *n*-hexane extract *of A. millefolium*, and antibacterial properties against phytopathogenic bacteria of *n*-hexane extracts and essential oils of *A. biebersteinii* and *A. millefolium*.

The main objectives of this study are to: (1) determine the chemical compositions of the essential oils and hexane extracts of the plant samples and the fractions of the *n*-hexane extract of *T. fallax* over silica gel column chromatography; (2) evaluate the antibacterial effects of *n*-hexane, chloroform, acetone and methanol extracts and hydrodistillated essential oils and/or their major components of *Satureja spicigera* (C. Koch) Boiss., *Thymus fallax* Fisch. & CA Mey, *Achillea biebersteinii* Afan, and *Achillea millefolium* L. on 25 phytopathogenic bacterial strains; and (3) determine if the essential oils of *S. spicigera*, thymol and

Table 2. Plant pathogenic bacterial species used in the study

	Bacterium	Host	Strain no
1	*Alcaligenes piechaudii*	Apple	RK-155
2	*Bacillus pumilus*	Apple	RK-106
3	*Choromobacterium violaceum*	Pear	RK-231
4	*Clavibacter michiganensis* ssp. *michiganensis*	Tomato	RK-Cmm
5	*Enterobacter intermedius*	Apple	RK-90
6	*Erwinia amylovora*	Pear	RK-228
7	*Erwinia carotovora* subsp. *atroceptica*	Potato	RK-462
8	*Erwinia chrysanthemi*	Potato	RK-421
9	*Erwinia rhapontici*	Pear	RK-208
10	*Flavobacter* sp.	Pear	RK-299
11	*Pantoea agglomerans*	Apple	RK-84
12	*Pseudomonas aeruginosa*	Apple	RK-168
13	*Pseudomonas cichorii*	Lettuce	RK-166
14	*Pseudomonas huttiensis*	Pear	RK-260
15	*Pseudomonas putida*	Pear	RK-249
16	*Pseudomonas syringae* pv. *syringae*	Apricot	RK-402
17	*Pseudomonas syringae* pv. *tomato*	Tomato	RK-Ps-tom
18	*Xanthomonas axanopodis* pv. *malvacearum*	Cotton	RK-Xa-mal
19	*Xanthomonas axanopodis* pv. *vesicatoria*	Tomato	Xcv761
20	*Xanthomonas axanopodis* pv. *vesicatoria*	Pepper	Xcv110c
21	*Xanthomonas axanopodis* pv. *campestris*	Cabbage	RK-Xa-cam
22	*Xanthomonas campestris* pv. *raphani*	Radish	RK-Xc-rap
23	*Xanthomonas axanopodis* pv. *vitians*	Lettuce	Xa-vit
24	*Xanthomonas campestris* pv. *zinniae*	Zinniae	Xc-zin
25	*Xanthomonas axanopodis* pv. *pelargonii*	Geranium	RK-Xa-pel

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Table 3. Chemical composition of the essential oils and hexane extracts of *S. spicigera* and *T. fallax*

		S. spicigera		*T. fallax*		
RI[a]	Component	EO (%)	HE (%)	EO (%)	HE (%)	Identification methods
938	α-Pinene	3.01	–	–	–	GC, MS, RI
983	β-Pinene	0.34	–	–	–	GC, MS, RI
995	Myrcene	1.92	–	–	–	GC, MS, RI
1014	3-Carene	0.34	–	–	–	GC, MS, RI
1023	α-Terpinene	2.81	–	–	–	GC, MS, RI
1034	*p*-Cymene	18.25	tr	2.36	7.65	GC, MS, RI
1042	1,8-Cineole	–	–	5.12	tr	GC, MS, RI
1045	(*E*)-β-Ocimene	0.49	–		–	GC, MS, RI
1067	γ-Terpinene	11.16	tr	1.12	7.32	GC, MS, RI
1106	Linalool	0.08	–	–	–	GC, MS, RI
1153	Camphor	–	–	1.60	tr	GC, MS, RI
1145	*trans*-Pinocarveol	0.09	–	–	–	GC, MS, RI
1150	*trans*-Verbenol	0.12	–	–	–	GC, MS, RI
1172	Borneol	0.30	–	3.60	2.34	GC, MS, RI
1178	Terpinen-4-ol	1.01	–	1.51	tr	GC, MS, RI
1185	*p*-Cymen-8-ol	0.08	–	–	–	GC, MS, RI
1190	α-Terpineol	0.41	1.04	0.90	tr	GC, MS, RI
1219	Thymol methyl ether	14.44	3.01	tr	tr	GC, MS, RI
1228	Carvacrol methyl ether	tr	tr	tr	tr	GC, MS, RI
1234	Pulegone	–	–	tr	–	GC, MS, RI
1267	Thymoquinone	0.10	1.79	tr	2.14	GC, MS, RI
1278	Bornyl acetate	–	–	tr	–	GC, MS, RI
1285	Thymol	23.99	47.25	4.29	13.24	GC, MS, RI
1296	Carvacrol	10.76	23.04	51.26	58.72	GC, MS, RI
1346	α-Terpinyl acetate	0.24	–	–	–	GC, MS, RI
1373	α-Copaene	0.08	–	–	tr	GC, MS, RI
1383	β-Bourbonene	0.17	–	1.01	tr	GC, MS, RI
1408	Citronellyl oxy-acetaldehyde	–	2.49	–	–	MS, RI
1419	β-Caryophyllene	4.37	–	5.25	1.50	GC, MS, RI
1453	(*Z*)-β-Farnesene	–	–	0.59	tr	GC, MS, RI
1460	α-Humulene	0.23	–	–	–	GC, MS, RI
1463	(*E*)-β-Farnesene	–	–	1.73	tr	GC, MS, RI
1478	γ-Muurolene	0.21	–	–	tr	GC, MS, RI
1486	Germacrene D	0.47	–	4.14	tr	GC, MS, RI
1501	Bicyclogermacrene	–	–	0.98	tr	GC, MS, RI
1494	Viridiflorene	0.24	–	–	–	GC, MS, RI
1507	(*E,E*)-α-Farnesene	–	–	1.10	1.05	GC, MS, RI
1508	β-Bisabolene	1.38	–	–	tr	MS, RI
1512	γ-Cadinene	0.57	–	5.13	tr	MS, RI
1515	Geranyl isobutanoate	–	2.85	–	0.46	MS, RI
1517	δ-Cadinene	–	–	–	tr	GC, MS, RI
1555	(*E*)-Nerolidol	–	–	0.94	tr	GC, MS, RI
1574	Spathulenol	0.45	1.70	1.45	1.02	GC, MS, RI
1579	Caryophyllene epoxide	0.51	4.60	1.32	1.45	GC, MS, RI
1625	1-*epi*-Cubenol	–	–	–	0.45	MS, RI
1659	α-Cadinol	–	–	2.43	tr	GC, MS, RI
1695	Shyobunol	–	–	2.16	tr	MS, RI
1908	Methyl palmitate	–	–	–	1.38	GC, MS, RI
2000	*n*-Eicosane	–	1.67	–	–	GC, MS, RI
2600	*n*-Hexacosane	–	–	–	2.55	GC, MS, RI
Grouped components (%)						
Aromatic monoterpenes		67.44	73.30	57.91	81.75	

Table 3. (*Continued*)

RI[a]	Component	*S. spicigera* EO (%)	*S. spicigera* HE (%)	*T. fallax* EO (%)	*T. fallax* HE (%)	Identification methods
Monoterpene hydrocarbons		20.07	tr	1.12	7.32	
Oxygenated monoterpenes		2.43	8.17	12.73	2.80	
Sesquiterpene hydrocarbons		8.25	–	19.93	2.55	
Oxygenated sesquiterpenes		096	6.30	8.30	2.92	
Others		–	–	–	1.38	
Total identified		99.15	87.77	99.99	98.72	

[a] Retention index relative to *n*-alkanes on SGE-BPX5 capillary column; GC, identification was based on retention times of authentic compounds on SGE-BPX5 capillary column; MS, identification was based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data;[30] RI, tentatively identified based on comparison of retention index of the compounds compared with published data.[30]
EO, essential oil; HE, *n*-hexane extract; tr, traces (less than 0.08%).

Table 4. Chemical composition of the fractions obtained by silica gel column chromatography of hexane extract of *T. fallax*

Component	%	Component	%
Fraction A		**Fraction C**	
α-Pinene	tr	*p*-Cymene	4.42
Myrcene	tr	Bornyl acetate	7.49
p-Cymene	44.58	Thymol	80.66
γ-Terpinene	13.87	α-Muurolene	1.68
Thymol methyl ether	tr	γ-Cadinene	tr
Carvacrol methyl ether	2.88	δ-Cadinene	4.34
α-Copaene	tr	Total identified (%)	98.59
β-Bourbonene	5.28		
β-Caryophyllene	13.66	**Fraction D**	
β-Gurjunene	tr	1,8-Cineole	2.00
(*Z*)-β-Farnesene	tr	Camphor	0.84
Alloaromadendrene	3.88	Borneol	tr
γ-Muurolene	2.51	Terpinen-4-ol	tr
Germacrene D	4.12	Thymol	9.86
β-Bisabolene	3.89	Carvacrol	86.78
γ-Cadinene	tr	Caryophyllene oxide	0.52
δ-Cadinene	5.32	Total identified (%)	100.00
Total identified (%)	99.99		
		Fraction E	
Fraction B		1,8-Cineole	9.51
p-Cymene	11.64	*cis*-Sabinene hydrate	7.00
Thymoquinone	4.79	*trans*-Sabinene hydrate	7.15
Carvacrol methyl ether	4.04	Borneol	30.51
Bornyl acetate	9.88	Terpinen-4-ol	7.47
Thymol	5.20	α-Terpineol	7.61
Carvacrol	tr	Thymol	5.34
1-Tetradecene	9.86	Carvacrol	9.38
n-Tetradecane	9.32	Spathulenol	8.48
δ-Cadinene	9.92	β-Eudesmol	7.26
Caryophyllene oxide	19.21	Total identified (%)	99.71
Methyl palmitate	3.28		
n-Heneicosane	5.88		
n-Hexacosane	4.22		
Total identified (%)	97.24		

tr, traces (less than 0.52%).

carvacrol could be used as potential seed disinfectant agents against *C. michiganensis* subsp. *michiganensis, P. syringae* pv. *tomato, X. axonopodis* pv. *vesicatoria* and *X. axonopodis* pv. *vitians.*

MATERIAL AND METHODS

Plant materials

The aerial parts of the plant samples were collected from the northern Anatolia region of Turkey at the flowering stages of the plant samples in July–September and were dried in shade. The voucher specimens have been deposited in the herbarium of Department of Plant Protection in Atatürk University, Erzurum, Turkey.

Isolation of the essential oils

The dried plant samples (500 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The oils were extracted with $CHCl_3$ and then were dried over anhydrous Na_2SO_4 and stored under N_2 atmosphere at 20 °C in a sealed vial until use. The essential oil yields, which were based on dry materials of the plant samples, are shown in Table 1.

Extraction procedures

The dried plant samples were powdered in a blender and then 50 g of each plant sample was 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 extracts of the plant samples, the concentrated MeOH extracts were individually dissolved in distilled water (60 °C) and then filtered. The solutions were extracted with *n*-hexane for three times to remove lipophilic compounds. Then, water solutions were lyophilised in a Labconco 117 freeze-dryer (Labconco Company, Kansas city, MO, USA) at 5 μm Hg and −50 °C. The extracts yields were shown in Table 1.

General experimental procedures

Column chromatography (CC) was carried out using silica gel 60 (70–230 and 200–400 mesh), thin layer chromatography (TLC) and preparative TLC on silica gel 60 precoated plates, F-254 (Merck,

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Table 5. Chemical composition of *n*-hexane extracts and essential oils of aerial parts of *Achillea* species

RI[a]	Components	*A. biebersteinii*		*A. millefolium*		Identification methods
		EO (%)	HE (%)	EO (%)	HE (%)	
928	Tricyclene	–	–	0.18	–	GC, MS, RI
938	α-Pinene	1.00	–	0.71	–	GC, MS, RI
957	Camphene	2.38	–	0.14	–	GC, MS, RI
979	Sabinene	–	–	0.44	–	GC, MS, RI
983	β-Pinene	1.13	–	0.78	–	GC, MS, RI
987	1-Octen-3-ol	–	–	0.10	–	GC, MS, RI
1012	α-Phellandrene	–	–	0.05	–	GC, MS, RI
1023	α-Terpinene	0.57	–	0.21	–	GC, MS, RI
1034	*p*-Cymene	1.74	–	0.92	–	GC, MS, RI
1042	1,8-Cineole	38.09	15.12	6.58	0.32	GC, MS, RI
1067	α-Terpinene	1.11	–	0.61	–	GC, MS, RI
1079	*cis*-Sabinenehydrate	tr	1.11	–	–	GC, MS, RI
1081	*cis*-Linalool oxide (furanoid)	tr	–		–	GC, MS, RI
1091	Terpinolene	tr	–	0.10	–	GC, MS, RI
1106	Linalool	0.66	1.53	0.51	–	GC, MS, RI
1113	Nonanal	–		0.11	–	GC, MS, RI
1114	*cis*-Thujone	tr	–	–	–	GC, MS, RI
1125	*trans*-Thujone	tr	–	0.14	–	GC, MS, RI
1130	*cis-p*-Menth-2-en-1-ol	tr	–	–	–	GC, MS, RI
1134	α-Campholenal	1.64	0.12	0.15	–	GC, MS, RI
1139	*cis*-Limonene oxide	–	–	0.82	–	GC, MS, RI
1145	*trans*-Pinocarveol	–	–	0.71	–	GC, MS, RI
1150	*trans*-Verbenol	0.89	–	0.97	–	GC, MS, RI
1153	Camphor	23.56	17.97	2.28	–	GC, MS, RI
1162	Sabinaketone	1.47	–	0.12	–	MS, RI
1172	Borneol	5.88	5.15	1.36	0.30	GC, MS, RI
1178	Terpinen-4-ol	3.26	–	2.96	–	GC, MS, RI
1185	*p*-Cymen-8-ol	tr	0.11	0.24	–	MS, RI
1190	α-Terpineol	5.15	0.60	3.48	–	GC, MS, RI
1191	Myrtenal	–	–	0.30	–	GC, MS, RI
1201	Verbenone	–	–	0.10	–	MS, RI
1211	*trans*-Carveol	tr	–	–	–	GC, MS, RI
1217	*cis*-Carveol	–	1.17	–	–	GC, MS, RI
1243	*trans*-Chrysanthenyl acetate	–	–	0.94	–	MS, RI
1234	Pulegone	–	–	1.70	–	GC, MS, RI
1242	Cumin aldehyde	0.53	–	–	–	GC, MS, RI
1254	Piperitone	0.31	1.03	1.10	–	GC, MS, RI
1255	Linalyl acetate	–	–	0.87	–	GC, MS, RI
1278	Bornyl acetate	1.35	0.66	0.56	–	GC, MS, RI
1285	Lavundulyl acetate	–	–	0.10	–	MS, RI

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Table 5. (*Continued*)

RI[a]	Components	*A. biebersteinii*		*A. millefolium*		Identification methods
		EO (%)	HE (%)	EO (%)	HE (%)	
1289	Thymol	0.98	1.73	3.95	4.81	GC, MS, RI
1296	Carvacrol	tr	1.26	1.04	4.68	GC, MS, RI
1332	*p*-Mentha-1,4-dien-7-ol	0.78	–	–	–	MS, RI
1337	*trans*-Carvyl acetate	–	–	0.07	–	GC, MS, RI
1345	Piperitenone	–	–	0.58	–	GC, MS, RI
1357	Eugenol	tr	–	0.18	–	GC, MS, RI
1360	*cis*-Carvyl acetate	–	–	0.05	–	GC, MS, RI
1369	Piperitenone oxide	–	–	0.22	–	GC, MS, RI
1373	α-Copaene	–	–	0.57	–	GC, MS, RI
1383	β-Bourbonene	–	–	0.39	–	MS, RI
1388	β-Elemene	–	–	0.15	–	MS, RI
1399	(*Z*)-Jasmone	0.26	–	0.08	–	GC, MS, RI
1413	4aα,7β,7aα-Nepetalactone	–	–	1.28	–	MS, RI
1419	β-Caryophyllene	0.43	–	3.24	–	GC, MS, RI
1433	β-Gurjunene	tr	–	0.12	–	GC, MS, RI
1442	Aromadendrene	–	–	0.21	–	GC, MS, RI
1453	(*Z*)-β-Farnesene	–	–	0.13	–	GC, MS, RI
1460	α-Humulene	–	–	1.04	–	GC, MS, RI
1464	*allo*-Aromadendrene	–	–	0.11	–	GC, MS, RI
1474	γ-Gurjunene	–	–	0.72	–	GC, MS, RI
1476	β-Chamigrene	0.28	–	–	–	MS, RI
1486	Germacrene D	3.00	–	1.62	–	GC, MS, RI
1496	α-Zingiberene	–	–	0.40	–	MS, RI
1509	Germacrene A	–	–	0.21	–	MS, RI
1513	γ-Acoradiene	–	–	0.18	–	MS, RI
1513	γ-Cadinene	–	–	0.17	–	MS, RI
1517	δ-cadinene	–	–	0.54	–	GC, MS, RI
1521	(*Z*)-Nerolidol	–	–	0.69	–	GC, MS, RI
1541	α-Calacorene	–	–	0.23	–	GC, MS, RI
1555	(*E*)-Nerolidol	–	–	2.81	–	GC, MS, RI
1574	Spathulenol	0.30	0.23	2.12	0.89	GC, MS, RI
1579	Caryophyllene epoxide	0.31	0.39	5.58	1.59	GC, MS, RI
1595	Viridiflorol	–	–	1.67	–	GC, MS, RI
1631	γ-Eudesmol	0.51	–	–	–	MS, RI
1636	δ-Cadinol	–	–	6.21	–	GC, MS, RI
1647	α-Bisabolol oxide B	–	–	0.35	1.05	MS, RI
1651	β-Eudesmol-45.28	0.95	–	1.36	1.57	MS, RI
1682	*epi*-α-Bisabolol	–	–	–	2.49	MS, RI

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Table 5. (*Continued*)

1687	Bisabolene oxide	–	–	1.58	–	MS, RI
1690	α-Bisabolol	–	–	2.85	–	MS, RI
1745	Chamazulene	–	–	0.23	–	GC, MS, RI
1760	α-Bisabolol oxide A	–	–	–	2.65	MS, RI
1816	Isolongifolol oxide	–	–	–	2.09	GC, MS, RI
1844	(*Z,Z*)-Farnesyl acetone	0.60	–	0.25	1.31	MS, RI
1849	*n*-Hexadecanol	tr	–	–	–	MS, RI
1908	Methyl palmitate	–	–	–	2.57	GC, MS, RI
1923	*n*-Hexadecanoic acid	0.31	–	–	1.03	GC, MS, RI
1957	Isophytol	–	–	–	3.84	GC, MS, RI
1955	Methyl linoleate	0.25	10.18	–	3.01	GC, MS, RI
1970	Linoleic acid	–	16.40	–	–	GC, MS, RI
2000	*n*-Eicosane	0.24	2.73	–	5.83	GC, MS, RI
2100	*n*-Heneicosane	0.24	7.23	–	8.95	GC, MS, RI
2300	*n*-Tricosane	–	9.84	–	10.28	GC, MS, RI
2400	*n*-Tetracosane	–	–	–	1.45	GC, MS, RI
2600	*n*-Hexacosane	–	–	–	23.21	GC, MS, RI
Grouped components (%)						
Monoterpene hydrocarbons		7.93	–	4.14	–	
Oxygenated monoterpenes		83.98	47.56	32.08	10.11	
Sesquiterpene hydrocarbons		3.71	–	10.26	–	
Oxygenated sesquiterpenes		2.67	0.62	26.75	13.64	
Others		1.04	46.38	0.29	60.17	
Total		99.33	94.56	73.52	83.92	

EO, essential oil. HE, *n*-hexane extract.
[a] Retention index relative to *n*-alkanes on SGE-BPX5 capillary column; GC, identification was based on retention times of authentic compounds on SGE-BPX5 capillary column; MS, tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data;[30] RI, identification was based on comparison of retention index with those of published data.[30] tr, traces (less than 0.05%).

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Darmstadt, Germany). The spots on TLC were visualised by ultraviolet light at 254 and 366 nm, and spraying with 1% vanillin–H_2SO_4 followed by heating (105 °C).

GC–FID Analysis

The analysis of the essential oil was performed using a Thermofinnigan Trace GC/A1300 (E.I.) (San Jose, USA) equipped with a SGE/BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm). Helium was the carrier gas, at a flow rate of 1 mL min^{-1}. The injector temperature was set at 220 °C. The oven temperature was 50–150 °C at a rate of 3 °C min^{-1}, then held isothermal for 10 min at 150 °C and finally raised to 250 °C at 10 °C min^{-1}. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected manually and in the splitless mode. Quantitative data were obtained from FID area percentage data.

GC–MS Analysis

The analysis of the essential oil was performed with 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 ionisation system with ionisation energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 mL min^{-1}. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The oven temperature was programmed from 50 °C to 150 °C at 3 °C min^{-1}, then held isothermal for 10 min and finally raised to 250 °C at 10 °C min^{-1}. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected manually in the splitless mode.

The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on SGE-BPX5 capillary column, and by matching their mass spectra of peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data.[30]

Fractionation of *n*-hexane extract of *T. fallax*

In order to fractionate the *n*-hexane extract of *T. fallax*, powdered aerial parts (180 g) of the plant sample was extracted with *n*-hexane (1 L × 5) at room temperature. After filtration, the extract was concentrated under vacuum using a rotary evaporator to yield 6.00 g extract. The concentrated hexane extract (6.00 g) was fractioned on silica gel CC (120 g, 70–230 mesh) using elution systems, $CHCl_3$: *n*-hexane (8 : 2), $CHCl_3$ and $CHCl_3$: acetone (8 : 2). A total of 60 fractions was collected and the fractions (25 mL each) were compared by TLC (silica gel) using $CHCl_3$: *n*-hexane (5 : 5, 6 : 4, 7 : 3), $CHCl_3$ and $CHCl_3$: ethyl acetate (8 : 2 and 7 : 3) and those giving similar spots were combined. Hence, five fractions (A–E) were finally obtained. The amounts of the fractions were 1240, 80, 64, 521 and 1757 mg for A, B, C, D and E, respectively.

Phytopathogenic bacterial strains

The essential oils and the extracts of the plant species, and six major monoterpenes were individually tested against 25 phytopathogenic bacterial strains (Table 2). The bacterial strains have been isolated from some fruits and vegetables exhibiting typical bacterial disease symptoms on their respective host plants. The bacteria were identified by using a microbial identification system (MIS).[31] Bacterial cultures were preserved in Luria broth and 15% glycerol solution at −80 °C prior to use.

Antibacterial activity assays

Antibacterial activity assays were carried out by disk diffusion method on tryptic soy agar (TSA; Merck) medium. The extracts, the fractions of *T. fallax* hexane extract and monoterpenes solutions were prepared by dissolving in suitable solvents, chloroform, acetone and water, and then were sterilised over filtration by 0.45 µm Millipore filters. Bacterial cultures were grown in tryptic soy broth (TSB; Merck), and their suspension (100 µL) containing 1×10^8 CFU mL^{-1} of bacteria spread by a sterile swab on TSA medium. The disks (6 mm in diameter) were impregnated with 12.5 µL of the essential oils and liquid monoterpenes, 1,8-cineole, carvacrol and α-terpineol, and 1.25 mg of the extracts, fractions and solid monoterpenes (borneol, camphor and thymol) solutions and the disks were kept out to evaporate the organic solvents. 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 the disks. Furthermore, bactericidal and bacteriostatic activities were also determined. The nutrient agar samples taken from inhibited areas around of the disks were put into nutrient broth without essential oil, extract and pure compounds, and incubated at 27 ± 2 °C for 48 h. After 48 h, if no bacterial growth was observed in the broth culture it was considered as bactericidal effect. If any bacterial growth was observed in the broth culture it was considered as bacteriostatic effect. All the tests were carried out in triplicate.

Determination of minimal inhibition concentration

MIC values were determined by using the modified agar-well diffusion method. Two-fold serial dilutions of the essential oils and carvacrol (500 µL mL^{-1}) were prepared by diluting 10% DMSO to achieve a decreasing concentration range from 500 µL mL^{-1} to 3.125 µL mL^{-1}. However, solutions of the extracts, the fractions of *T. fallax* and thymol and α-terpineol were prepared at concentrations range from 10 to 100 mg mL^{-1}. A 100 µL of bacterial suspension containing 1×10^8 CFU mL^{-1} of bacteria was spread on TSA plates. The disks were impregnated with 12.5 µL of the solutions tested. Then, they were put in the middle of inoculated TSA agar plates. The bacterial cultures were incubated at 27 ± 2 °C for 48 h. The least concentration of each solution showing a clear zone of inhibition was taken as the MIC value. All the tests were carried out in triplicate.

Seed disinfection assays

Tomato, pepper and lettuce seeds (Marmande, A36D1) were purchased from a local market and surface disinfected to avoid the presence of any saprobic and/or pathogenic microorganisms on the seed surface. Seed disinfection was performed by dipping the seeds for 3 min in 3% sodium hypochlorite and washing four times in sterile distilled water. Seeds were left to dry on sterile Whatman filter paper sheets overnight in a laminar flow hood for using further studies, and thereafter pathogenic bacterial strains *C. michiganensis* subsp. *michiganensis, P. syringae* pv. *tomato, X. axonopodis* pv. *vesicatoria* and *X. axonopodis* pv. *vitians* were grown in 50 mL Erlenmeyer flasks containing 20 mL of TSB medium on a rotary shaker at 28 °C for 24 h. Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and appropriately diluted to 1×10^8 CFU mL^{-1} in sdH_2O. Approximately 20 g of sucrose (10 g L^{-1}) was added to each Erlenmeyer flask, and 90 g of the surface-sterilised seeds were soaked separately in this suspension. The seeds were incubated in Erlenmeyer flasks

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Table 6. Antibacterial activities of the essential oil and the extracts of *S. spicigera*

	Essential oil		Extracts						
			Hexane		$CHCl_3$		Acetone		Methanol
Bacterium	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD
A. piechaudii RK-155	15*	31.25	8*	90.0	–	ND	–	ND	–
B. pumilus RK-106	48*	125.0	–	ND	–	ND	9*	90.0	–
C. violaceum RK-231	19	31.25	–	ND	–	ND	–	ND	–
C. michiganensis subsp. *michiganensis* Cmm	55*	31.25	11*	70.0	8*	70.0	13*	80.0	–
E. intermedius RK-90	16*	62.50	8*	90.0	8*	90.0	–	ND	–
E. amylovora RK-228	37*	125.0	9*	70.0	9*	90.0	14*	90.0	–
E. caratovora subsp. *atroceptica* RK-462	26*	31.25	10*	90.0	–	ND	–	ND	–
E. chrysanthemi RK-421	16	31.25	8*	90.0	10*	90.0	–	ND	–
E. rhapontici RK-208	28*	62.50	9*	90.0	8*	90.0	9*	90.0	–
Flavobacter sp. RK-299	47	31.25	17	60.0	16	70.0	22*	70.0	–
P. agglomerans RK-84	14*	62.50	8*	90.0	–	ND	–	ND	–
P. aeruginosa RK-168	18*	62.50	–	ND	–	ND	–	ND	–
P. cichorii RK-166	19*	62.50	–	ND	–	ND	–	ND	–
P. huttiensis RK-260	46	62.50	10*	70.0	12*	80.0	20	70.0	–
P. putida RK-249	9*	31.25	–	ND	–	ND	–	ND	–
P. syringae pv. *syringae* RK-204	37	31.25	9*	70.0	13*	80.0	11*	80.0	–
P. syringae pv. *tomato* RK-Ps-tom	21	31.25	10*	70.0	10*	70.0	10*	90.0	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	50*	31.25	11*	70.0	9*	80.0	10*	80.0	–
X. axonopodis pv. *vesicatoria* Xcv110c	29	31.25	11*	70.0	–	ND	9*	90.0	–
X. axonopodis pv. *campestris* RK-Xa-cam	29	15.63	9*	80.0	8*	80.0	8*	90.0	–
X. campestris pv. *raphani* RK-Xc-rap	54*	62.50	10*	70.0	10*	80.0	11*	80.0	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	23	31.25	8*	80.0	8*	90.0	–	ND	–
X. axonopodis pv. *vitians* Xa-vit	38	31.25	9*	80.0	9*	80.0	9	80.0	–
X. campestris pv. *zinniae* Xc-zin	28*	62.50	9*	90.0	–	ND	–	ND	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	42	31.25	11*	70.0	9*	80.0	11*	80.0	–

DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 μL of the essential oil; MIC, minimal inhibitory concentration as $\mu L\ mL^{-1}$ for the essential oil and in $mg\ mL^{-1}$ for the extracts; ND, MIC value was not determined.
* Bactericidal effect was observed; – , not active.

by shaking at 80 rpm for 2 days at 28 °C to coat the seeds with pathogen. After shaking, the seeds were taken out and air-dried on sterile Whatman filter paper sheets overnight in the laminar flow hood.

Suitable concentrations (100, 40, 20, 10, 4 and 2 $mg\ mL^{-1}$) of *S. spicigera* oil, carvacrol and thymol were selected by doing preliminary experiments as MIC values. Prior to bioassays, the solutions were prepared by dissolving 10% DMSO–water (v/v) in 10 mL Erlenmeyer flasks. The seeds coated with pathogen were soaked in the suspensions, and incubated by shaking at 80 rpm for 1 day at 28 °C until the seeds were uniformly coated with the suspensions. Seeds were left to dry on sterile Whatman no. 1 filter paper sheets overnight in the laminar flow hood. Streptomycin sulfate (0.5 $mg\ mL^{-1}$) and hot water (52 °C) were used as positive controls, whereas 10% DMSO–water solution without essential oil, carvacrol and thymol was used as a negative control. The Petri dishes containing 20 seeds and 20 mL of TSA medium were incubated at 27 ± 2 °C. After 6 days, the number of seeds infected or not infected with pathogenic bacteria was counted. If no pathogenic bacterial growth was detected on the plates, the seed samples were considered as sterile. The treatments were arranged in a completely randomised design with six replications for each treatment, including controls.

Statistical analyses

In order to determine whether there is a statistically significant difference between the obtained results for seed disinfectant assays, variance analyses were carried out using SPSS 10.0 software package. Differences between means were tested by Duncan's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Chemical composition of the essential oils and *n*-hexane extracts

The yields of essential oils and extracts isolated from the plant species used in this study were given in Table 1. The chemical compositions of the essential oils and hexane extracts of *S. spicigera* and *T. fallax* were given in Table 3. In general, the essential oils have similar chemical composition. Thymol (23.99%), *p*-cymene (18.25%), γ-terpinene (11.16%) and thymol methyl ether (14.44%) were the major components of *S. spicigera* oil, whereas *T. fallax* oil contained mainly carvacrol (51.26%), β-caryophyllene (5.25%), γ-cadinene (5.13%), 1,8-cineole (5.12%) and thymol (4.29%) (Table 3). As shown in Table 3, these essential oils also contain relatively high contents of oxygenated monoterpenes, representing 69.87% and 70.64% of the oils, respectively.

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Table 7. Antibacterial activities of the essential oil and extracts of *T. fallax*

	Essential oil		Extracts						
			Hexane		$CHCl_3$		Acetone		Methanol
Bacterium	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD
A. piechaudii RK-155	12	31.25	–	ND	–	ND	–	ND	–
B. pumilus RK-106	18*	31.25	–	ND	–	ND	–	ND	–
C. violaceum RK-231	24*	31.25	–	ND	–	ND	–	ND	–
C. michiganensis subsp. *michiganensis* Cmm	24	62.50	11*	80.0	–	ND	10*	70.0	–
E. intermedius RK-90	20	62.50	–	ND	–	ND	–	ND	–
E. amylovora RK-228	36	31.25	–	ND	–	ND	8*	90.0	–
E. caratovora subsp. *atroceptica* RK-462	33*	31.25	–	ND	–	ND	–	ND	–
E. chrysanthemi RK-421	19	31.25	–	ND	–	ND	–	ND	–
E. rhapontici RK-208	30	31.25	–	ND	8*	90.0	–	ND	–
Flavobacter sp. RK-299	42*	15.63	15*	70.0	8*	80.0	12	80.0	–
P. agglomerans RK-84	18	31.25	–	ND	–	ND	–	ND	–
P. aeruginosa RK-168	18	125.0	–	ND	–	ND	–	ND	–
P. cichorii RK-166	17*	125.0	–	ND	–	ND	–	ND	–
P. huttiensis RK-260	27	31.25	–	ND	–	ND	10*	70.0	–
P. putida RK-249	10*	250.0	–	ND	–	ND	–	ND	–
P. syringae pv. *syringae* RK-204	30	31.25	–	ND	–	ND	–	ND	–
P. syringae pv. *tomato* RK-Ps-tom	28	62.50	8*	90.0	–	ND	–	ND	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	43	31.25	–	ND	–	ND	10*	80.0	–
X. axonopodis pv. *vesicatoria* Xcv110c	41	31.25	–	ND	–	ND	–	ND	–
X. axonopodis pv. *campestris* RK-Xa-cam	42*	15.63	–	ND	–	ND	–	ND	–
X. campestris pv. *raphani* RK-Xc-rap	38*	31.25	8*	90.0	–	ND	8*	80.0	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	32	31.25	–	ND	–	ND	10*	80.0	–
X. axonopodis pv. *vitians* Xa-vit	41*	31.25	–	ND	–	ND	–	ND	–
X. campestris pv. *zinniae* Xc-zin	27	62.50	–	ND	–	ND	–	ND	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	45	15.63	8*	90.0	–	ND	–	ND	–

DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 μL of the essential oil; MIC, minimal inhibitory concentration as $\mu L\ mL^{-1}$ for the essential oil and in $mg\ mL^{-1}$ for the extracts.
* Bactericidal effect was observed; –, not active; ND, MIC value was not determined.

In the present study, the chemical composition of the *n*-hexane extracts of *S. spicigera* and *T. fallax* were analysed by GC–FID and GC–MS for the first time (Table 3). Thirteen compounds were detected in the hexane extract of *S. spicigera*, and thymol (47.25%), carvacrol (23.04%), caryophyllene epoxide (4.60%) and thymol methyl ether (3.01%) were found to be major components of the extract. However, citronellyl oxy-acetaldehyde (2.49%), geranyl isobutanoate (2.85%) and *n*-eicosane (1.67%) were not detected in the essential oil of *S. spicigera*. Similar to the chemical composition of the essential oil of *T. fallax*, its hexane extract consists of carvacrol (58.72%), thymol (13.24%), *p*-cymene (7.65%), γ-terpinene (7.32%), *n*-hexacosane (2.55%) and thymoquinone (2.14%) as predominant components (Table 3). However, γ-cadinene and germacrene D, which are the main components of *T. fallax* essential oil, were detected in trace amount in hexane extract (Table 3).

It has been shown that the essential oils of the genus *Satureja* and *Thymus* are rich in carvacrol, thymol, γ-terpinene and *p*-cymene.[7,17–20,32] *S. spicigera* from different regions of the world are divided into 'carvacrol type' and 'thymol type'. For instance, it has been reported that Iranian *S. spicigera* essential oil is thymol type.[17,18] On the other hand, relatively higher amounts of thymol than carvacrol have been found in the essential oil of *S. spicigera* from different localities in Turkey.[32] Our results showed that there were no significant differences in terms of thymol and carvacrol contents in *S. spicigera* oil (Table 3). However, it has been documented that Turkish *S. spicigera* collected from Artvin region in Turkey contained mainly carvacrol (42.5%), whereas thymol was detected in a small amount (0.3%).[19] As mentioned before, the essential oil content may be affected by local, climatic and seasonal factors. For example, severe water stress was reported to alter carvacrol/γ-terpinene contents.[33] The essential oils of *T. fallax* from Iran and Turkey were characterised by a high content of carvacrol (30.2–68.1%) and low amount of thymol.[14,15] In accordance with these findings, the essential oil of *T. fallax* contains mainly carvacrol (51.26%) and a relatively low amount of thymol (4.29%). However, *T. fallax* oil from different localities in Iran was characterised by high content of thymol (65.9%) as compared with carvacrol content.[16]

The hexane extract of *T. fallax* was subjected to silica gel CC using $CHCl_3$:*n*-hexane, $CHCl_3$ and $CHCl_3$:acetone (8 : 2) elution systems. The collected fractions were compared by TLC (silica gel) and those giving similar spots were combined. Hence, the hexane extract was fractionated into five fractions (A–E) and chemical compositions of these fractions were determined by GC–FID and GC–MS (Table 4). Fractions A and B contained generally terpene hydrocarbons as well as terpene ethers as the main components, such as *p*-cymene (44.58% vs. 11.64%), γ-terpinene (13.87% vs. 13.87%), β-caryophyllene (13.66% vs. not detected), δ-cadinene (5.32% vs.

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Table 8. Antibacterial activities of the major components of the essential oils tested

	Carvacrol		Thymol		Borneol		Camphor		1,8-Cineole		α-Terpineol	
Bacterium	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
A. piechaudii RK-155	16*	15.63	17*	20.0	–	ND	–	ND	–	ND	–	ND
B. pumilus RK-106	14*	15.63	22	20.0	–	ND	–	ND	–	ND	–	ND
C. violaceum RK-231	12*	31.25	17*	20.0	–	ND	–	ND	–	ND	–	ND
C. michiganensis subsp. *michiganensis* Cmm	23	31.25	41*	20.0	–	ND	–	ND	–	ND	–	ND
E. intermedius RK-90	16*	15.63	17*	20.0	–	ND	–	ND	–	ND	–	ND
E. amylovora RK-228	28	31.25	26*	20.0	–	ND	–	ND	–	ND	–	ND
E. caratovora subsp. *atroceptica* RK-462	15*	15.63	21*	20.0	–	ND	–	ND	–	ND	–	ND
E. chrysanthemi RK-421	16*	15.63	20*	20.0	–	ND	–	ND	–	ND	–	ND
E. rhapontici RK-208	23	31.25	24*	10.0	–	ND	–	ND	–	ND	–	ND
Flavobacter sp. RK-299	39	15.63	49*	20.0	–	ND	–	ND	–	ND	–	ND
P. agglomerans RK-84	19	15.63	15*	20.0	–	ND	–	ND	–	ND	–	ND
P. aeruginosa RK-168	13*	31.25	20*	20.0	–	ND	–	ND	–	ND	–	ND
P. cichorii RK-166	12*	31.25	21*	20.0	–	ND	–	ND	–	ND	8	70.0
P. huttiensis RK-260	23	15.63	31*	20.0	–	ND	–	ND	–	ND	10	60.0
P. putida RK-249	17	62.50	9*	40.0	–	ND	–	ND	–	ND	–	ND
P. syringae pv. *syringae* RK-204	26*	15.63	29*	20.0	–	ND	–	ND	–	ND	10	60.0
P. syringae pv. *tomato* RK-Ps-tom	25	15.63	26	20.0	–	ND	–	ND	–	ND	–	ND
X. axonopodis pv. *malvacearum* RK-Xa-mal	38	15.63	34	20.0	–	ND	–	ND	–	ND	–	ND
X. axonopodis pv. *vesicatoria* Xcv110c	28	15.63	28	20.0	–	ND	–	ND	–	ND	8	70.0
X. axonopodis pv. *campestris* RK-Xa-cam	25	15.63	29	20.0	–	ND	–	ND	–	ND	–	ND
X. campestris pv. *raphani* RK-Xc-rap	30	15.63	26	20.0	–	ND	–	ND	–	ND	–	ND
X. axonopodis pv. *vesicatoria* RK-Xcv761	32	15.63	26	20.0	–	ND	–	ND	–	ND	–	ND
X. axonopodis pv. *vitians* Xa-vit	28	15.63	38*	10.0	–	ND	–	ND	–	ND	–	ND
X. campestris pv. *zinniae* Xc-zin	20*	31.25	33	20.0	–	ND	–	ND	–	ND	–	ND
X. axonopodis pv. *pelargonii* RK-Xa-pel	29	15.63	30	20.0	–	ND	–	ND	–	ND	–	ND

DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; MIC, minimal inhibitory concentration as $\mu L\ mL^{-1}$ for the essential oil and in $mg\ mL^{-1}$ for the extracts.
* Bactericidal effect was observed; – , not active; ND, MIC value was not determined.

9.92%), β-bourbonene (5.28% vs. not detected), germacrene D (4.12% vs. not detected), *n*-tetradecane (not detected vs. 9.32%) and 1-tetradecene (not detected vs. 9.86%), respectively. However, caryophyllene epoxide (19.21%) was found to be main component of the fraction B. As can be seen from Table 4, fraction C contains thymol (80.66%) as main component, whereas fraction D contains mainly carvacrol (86.78%). On the other hand, fraction E contained mainly oxygenated monoterpenes such as borneol (30.51%), carvacrol (9.38%), 1,8-cineole (9.51%), β-eudesmol (7.26%), α-terpineol (7.61%) (Table 4).

The chemical compositions of the hydrodistillated essential oils and of hexane extracts isolated from the aerial parts of *A. biebersteinii* and *A. millefolium* were presented in Table 5. The chemical composition of the hexane extract of *A. millefolium* was reported for the first time in this study. The oil and hexane extract of *A. biebersteinii* were similar in terms of major monoterpene constituents, and 1,8-cineole, camphor, borneol and α-terpineol were found to be predominant components in the oil and extract (Table 5). However, hexane extract of *A. biebersteinii* were characterised relatively high amount of less volatile components, methyl linoleate (10.18%), linoleic acid (16.40%), *n*-tricosane (9.84%), *n*-eicosane (2.73%) and *n*-heneicosane (7.23%) (Table 5). These compounds were found to be lower in *A. biebersteinii* oil. On the other hand, the oil of *A. millefolium* contains relatively low amounts of 1,8-cineole (6.58%) and camphor (2.28%) as compared to *A. biebersteinii* oil. δ-Cadinol (6.21%), caryophyllene oxide (5.58%), thymol (3.95%), α-terpineol (3.48%), β-caryophyllene (3.24%) and terpinen-4-ol (2.96%) are other major components of *A. millefolium* oil. Furthermore, *A. biebersteinii* oil is rich in oxygenated monoterpenes, which comprises 83.98% of the oil as compared to *A. millefolium* oil (32.96%). In general, hexane extract of *A. millefolium* exhibited different chemical composition as compared to its essential oil (Table 5) and it was characterised by relatively high amount of less volatile components, *n*-hexacosane (23.21%), *n*-tricosane (10.28%), *n*-heneicosane (8.95%), *n*-eicosane (5.83%), methyl linoleate (3.01%) and methyl palmitate (2.57%). Furthermore, some compounds in the hexane extract of *A. millefolium* such as *epi*-α-bisabolol, α-bisabolol oxide, isolongifolol oxide and isophytol were not detected in the *A. millefolium* essential oil (Table 5).

Previous research revealed that essential oils isolated from some *Achillea* species growing in different regions of the world were characterised by their high content of 1,8-cineole, camphor, borneol and α-terpineol.[25,26,29] The chemical composition of *A. biebersteinii* essential oil and hexane extracts are compatible with the previous findings. However, in the current study, *A. millefolium* oil contained relatively low amounts of these compounds (Table 5).

Antibacterial activities of the essential oils, the extracts of the plant samples and pure components

In the present study, antibacterial activities of hydrodistillated essential oils, and the extracts isolated from the aerial parts of

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Table 9. Antibacterial activities of the fractions obtained by silica gel column chromatography from hexane extract of *T. fallax*

	A	B	C		D		E
Bacterium	DD	DD	DD	MIC	DD	MIC	DD
A. piechaudii RK-155	–	–	8*	80.0	12*	17.5	–
B. pumilus RK-106	–	–	9*	80.0	11*	17.5	–
C. violaceum RK-231	–	–	10*	80.0	10*	20.0	–
C. michiganensis subsp. *michiganensis* Cmm	–	–	9	90.0	23	50.0	–
E. intermedius RK-90	–	–	8*	80.0	12*	17.5	–
E. amylovora RK-228	–	–	9*	80.0	17	35.0	–
E. caratovora subsp. *atroceptica* RK-462	–	–	8*	80.0	14	17.5	–
E. chrysanthemi RK-421	–	–	8*	80.0	11*	17.5	–
E. rhapontici RK-208	–	–	8*	80.0	16	17.5	–
Flavobacter sp. RK-299	–	–	10*	70.0	24	17.5	–
P. agglomerans RK-84	–	–	8*	80.0	12	17.5	–
P. aeruginosa RK-168	–	–	–	ND	10*	35.0	–
P. cichorii RK-166	–	–	–	ND	9*	35.0	–
P. huttiensis RK-260	–	–	8*	80.0	23	8.75	–
P. putida RK-249	–	–	–	ND	16	35.0	–
P. syringae pv. *syringae* RK-204	–	–	8*	80.0	19	17.5	–
P. syringae pv. *tomato* RK-Ps-tom	–	–	8*	80.0	16*	20.0	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	–	–	8*	90.0	21	17.5	–
X. axonopodis pv. *vesicatoria* Xcv110c	–	–	17	80.0	26	17.5	–
X. axonopodis pv. *campestris* RK-Xa-cam	–	–	17	70.0	29	15.0	–
X. campestris pv. *raphani* RK-Xc-rap	–	–	11*	80.0	31	8.75	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	–	–	15	80.0	34	17.5	–
X. axonopodis pv. *vitians* Xa-vit	–	–	10*	80.0	28	17.5	–
X. campestris pv. *zinniae* Xc-zin	–	–	10	90.0	12	35.0	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	–	–	10*	70.0	26	17.5	–

DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; MIC, minimal inhibitory concentration as $\mu L\ mL^{-1}$ for the essential oil and in $mg\ mL^{-1}$ for the extracts.
* Bactericidal effect was observed; – , not active; ND, MIC value was not determined.

the plant species studied with *n*-hexane, $CHCl_3$, acetone and methanol were tested against 25 plant pathogenic bacterial strains (Tables 6–9). Our results showed that essential oils (12.5 $\mu L\ disk^{-1}$) of *S. spicigera* and *T. fallax* exhibited a broad spectrum of potent antibacterial activity against all tested bacterial strains producing 9–54 mm inhibition zones depending on the bacterial strains tested (Tables 6 and 7). The results presented in Tables 6 and 7 show that the MIC values of the oils vary with the bacterial strain tested, ranging from 15.63 to 125.0 $\mu L\ mL^{-1}$. Furthermore, *S. spicigera* oil had bactericidal activity against 14 bacterial strains tested (Table 6), whereas *T. fallax* oil possessed bactericidal activity against 10 bacterial strains (Table 7). Numerous reports indicated that essential oils containing mainly aromatic oxygenated monoterpenes, carvacrol and thymol, possessed the highest antimicrobial activity.[6–10,20,21] As shown in Table 3, the essential oils of *S. spicigera* and *T. fallax* consist mainly of carvacrol and thymol, which account for 55.55% and 34.75% of the essential oils, respectively. Therefore, high antibacterial activity of the essential oils of *S. spicigera* and *T. fallax* can be due to the high amount of carvacrol and thymol. The results presented in Table 8 also confirm these findings. As can be seen from this table, carvacrol and thymol exhibited potent antibacterial activity against all tested bacterial strains. On the basis of these results, it is clear that carvacrol and thymol, and the essential oils containing high amount of these components have potent antibacterial effect against tested bacteria. As to the mechanism of action of these agents, they cause alterations in the hyphal morphology and hyphal aggregates, resulting in reduced hyphal diameters and lysis of the hyphal wall interacting with the cell membrane of the pathogen.[34] However, it is also important to note that minor components as well as possible interactions among the constituents could affect the antibacterial activity of essential oils.

This study is the first to report the antibacterial properties of the hexane extracts of *S. spicigera* and *T. fallax* against 25 phytopathogenic bacterial strains (Tables 6 and 7). The hexane extract of *S. spicigera* showed similar antibacterial properties against the tested bacterial strains as compared to its essential oil (Table 6). However, the antibacterial effect of *S. spicigera* was found to be low as compared to the essential oil of *S. spicigera* with regard to low inhibition zones and high MIC values. As can be seen from Table 6, the hexane extract of this plant sample was not active against *B. pumilus, C. violaceum, P. aeruginosa, P. cichorii* and *P. putida*, whereas the essential oil of *S. spicigera* was effective against these bacteria. Similarly to the essential oil, thymol, (47.25%), carvacrol (23.04%), caryophyllene epoxide (4.60%) and thymol methyl ether (3.01%) are predominant components of hexane extract of *S. spicigera* (Table 3). However, as can be seen from Table 3, the essential oil contains many more minor terpenic compounds as compared to the hexane extract. Therefore, a low antibacterial effect of the hexane extract of *S. spicigera* can be attributed to the lack of some minor components. In contrast to the hexane extract of *S. spicigera*, the hexane extract of *T. fallax* showed

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Table 10. Antibacterial activities of the essential oil and extracts of *A. biebersteinii*

Bacterium	Essential oil		Extracts: Hexane	Extracts: $CHCl_3$	Extracts: Acetone	Extracts: Methanol
	DD	MIC	DD	DD	DD	DD
A. piechaudii RK-155	9*	250.0	–	–	–	–
B. pumilus RK-106	8*	500.0	–	–	–	–
C. violaceum RK-231	–	ND	–	–	–	–
C. michiganensis subsp. *michiganensis* Cmm	–	ND	–	–	–	–
E. intermedius RK-90	10*	250.0	–	–	–	–
E. amylovora RK-228	–	ND	–	–	–	–
E. caratovora subsp. *atroceptica* RK-462	10*	250.0	–	–	–	–
E. chrysanthemi RK-421	9*	250.0	–	–	–	–
E. rhapontici RK-208	13*	250.0	–	–	–	–
Flavobacter sp. RK-299	11*	125.0	–	–	–	–
P. agglomerans RK-84	9*	125.0	–	–	–	–
P. aeruginosa RK-168	11*	250.0	–	–	–	–
P. cichorii RK-166	11*	250.0	–	–	–	–
P. huttiensis RK-260	–	ND	–	–	–	–
P. putida RK-249	–	ND	–	–	–	–
P. syringae pv. *syringae* RK-204	16*	250.0	–	–	–	–
P. syringae pv. *tomato* RK-Ps-tom	9*	250.0	–	–	–	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	13	125.0	–	–	–	–
X. axonopodis pv. *vesicatoria* Xcv110c	9*	250.0	–	–	–	–
X. axonopodis pv. *campestris* RK-Xa-cam	12*	250.0	–	–	–	–
X. campestris pv. *raphani* RK-Xc-rap	12*	250.0	–	–	–	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	16	125.0	–	–	–	–
X. axonopodis pv. *vitians* Xa-vit	11*	125.0	–	–	–	–
X. campestris pv. *zinniae* Xc-zin	8*	250.0	–	–	–	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	16	125.0	–	–	–	–

DD, Inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; MIC, minimal inhibitory concentration as $\mu L\ mL^{-1}$ for the essential oil and in $mg\ mL^{-1}$ for the extracts.
* Bactericidal effect was observed; – , Not active; ND, MIC value was not determined.

bactericidal activity against seven bacteria, producing inhibition zones of 8–11 mm diameter and MIC values of 80–90 $mg\ mL^{-1}$ (Table 7). On the other hand, the hexane extract of *T. fallax* contains similar components as compared to its essential oil (Table 3). In the view of the present results, a high antibacterial effect of *T. fallax* essential oil may be due to some major and/or minor components in the oil. Weak antibacterial effects of the hexane extracts of *S. spicigera* and *T. fallax* may also be due to some components such as sterols, triterpenes, methoxylated flavonoid aglycones in the hexane extracts.

Furthermore, the hexane extract of *T. fallax* was fractionated by silica gel CC into five fractions (A–E) and these fractions were tested against 27 phytopathogenic bacterial strains (Table 9). Among these fractions, the C and D fractions were found to be active against the tested bacteria. In particular, fraction D, which contains mainly carvacrol (86.78%) and thymol (9.86%), was very effective against the tested bacterial strains. Our results also confirmed that the potent antibacterial effect of the essential oils of *Satureja, Thymus, Origanum* and *Thymbra* species is probably related to its carvacrol and thymol constituents. As can be seen from Table 9, fractions A, B and E, which contain low amounts or none of these components (Table 3), did not show any antibacterial activity against tested bacteria.

Besides the hexane extract of *S. spicigera* and *T. fallax*, $CHCl_3$, acetone and methanol extracts of these plant samples were tested for antibacterial properties against the bacterial strains tested. In particular, chloroform and acetone extracts of *S. spicigera* showed a varying antibacterial effect against the bacteria (Table 6). However, the chloroform and acetone extracts of *T. fallax* had antibacterial effects against a limited number of the bacteria (Table 7). In contrast, the methanol extracts of both plant species did not show any antibacterial effect.

The essential oils of *A. biebersteinii* and *A. millefolium* showed a broad spectrum of weak antibacterial activity against the tested bacteria (Tables 10 and 11). As can be seen from Table 5, *A. biebersteinii* and *A. millefolium* oils contain 1,8-cineole, camphor, borneol, α-terpineol and thymol as the major oxygenated monoterpenes. In the present study, some major components such as camphor, 1,8-cineole, borneol and α-terpineol were also tested for antibacterial activity against the phytopathogenic bacteria, and among these compounds, α-terpineol exhibited antibacterial activity against only five bacteria tested (Table 8). Similar results have been reported previously.[9,11,25] Therefore, the antibacterial effects of *A. biebersteinii* and *A. millefolium* oils can not be attributed to these major components. On the other hand, it should be considered that minor components in the essential oils, as well as synergistic and/or antagonistic interactions between

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Table 11. Antibacterial activities of the essential oil and extracts of *A. millefolium*

Bacterium	Essential oil		Extracts			
			Hexane	$CHCl_3$	Acetone	Methanol
	DD	MIC	DD	DD	DD	DD
A. piechaudii RK-155	7*	ND	–	–	–	–
B. pumilus RK-106	8*	250.0	–	–	–	–
C. violaceum RK-231	9*	250.0	–	–	–	–
C. michiganensis subsp. *michiganensis* Cmm	22	62.5	–	–	–	–
E. intermedius RK-90	–	ND	–	–	–	–
E. amylovora RK-228	15	250.0	–	–	–	–
E. caratovora subsp. *atroceptica* RK-462	–	ND	–	–	–	–
E. chrysanthemi RK-421	–	ND	–	–	–	–
E. rhapontici RK-208	19*	125.0	–	–	–	–
Flavobacter sp. RK-299	16*	125.0	–	–	–	–
P. agglomerans RK-84	8*	250.0	–	–	–	–
P. aeruginosa RK-168	–	ND	–	–	–	–
P. cichorii RK-166	–	ND	–	–	–	–
P. huttiensis RK-260	11*	250.0	–	–	–	–
P. putida RK-249	–	ND	–	–	–	–
P. syringae pv. *syringae* RK-204	10*	125.0	–	–	–	–
P. syringae pv. *tomato* RK-Ps-tom	9*	250.0	–	–	–	–
X. axonopodis pv. *malvacearum* RK-Xa-mal	13*	250.0	–	–	–	–
X. axonopodis pv. *vesicatoria* Xcv110c	11*	250.0	–	–	–	–
X. axonopodis pv. *campestris* RK-Xa-cam	18	125.0	–	–	–	–
X. campestris pv. *raphani* RK-Xc-rap	12*	125.0	–	–	–	–
X. axonopodis pv. *vesicatoria* RK-Xcv761	9*	125.0	–	–	–	–
X. axonopodis pv. *vitians* Xa-vit	9*	250.0	–	–	–	–
X. campestris pv. *zinniae* Xc-zin	9*	125.0	–	–	–	–
X. axonopodis pv. *pelargonii* RK-Xa-pel	12*	125.0	–	–	–	–

DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 µL of the essential oil; MIC, Minimal inhibitory concentration as $\mu L\ mL^{-1}$ for the essential oil and in $mg\ mL^{-1}$ for the extracts.
* Bactericidal effect was observed; – , not active; ND, MIC value was not determined.

the components could also affect the antibacterial effects of the essential oils.

Nevertheless, the hexane extracts of *A. biebersteinii* and *A. millefolium* were not effective against the tested bacteria. The oils and hexane extracts of *A. biebersteinii* were similar in terms of major monoterpene constituents, and 1,8-cineole, camphor, borneol, α-terpineol were found to be predominant components in the oil and extract (Table 5). However, hexane extracts of *A. biebersteinii* and *A. millefolium* were characterised by relatively high amounts of less volatile components, methyl linoleate, linoleic acid, *n*-tricosane, *n*-eicosane, *n*-heneicosane and *n*-hexacosane (Table 5). Therefore, no antibacterial activities of the hexane extracts of *A. biebersteinii* and *A. millefolium* can be attributed to their relatively high amounts of these components.

Seed disinfection

C. michiganensis subsp. *michiganensis, P. syringae* pv. *tomato, X. axonopodis* pv. *vesicatoria* and *X. axonopodis* pv. *vitians* are seed-borne pathogens that causes serious economic losses to both glasshouse and field cultivation of some vegetable plants such as tomato, pepper and lettuce in the world.[1,35] Therefore, the use of healthy seeds is a very important consideration in controlling seed-borne diseases. The results of seed disinfection assays are given in Table 12. Our results show that the essential oil of *S. spicigera*, carvacrol and thymol could be used as organic seed disinfection agents against the tested bacteria. However, the low concentrations of thymol and carvacrol were not in disinfecting pepper seeds infected with *X. axonopodis* pv. *vesicatoria*. These results indicated that the chemicals can display different activities on different host plants against the same pathogen. Furthermore, in the current study, the essential oils of *S. spicigera*, carvacrol and thymol were more effective than that of streptomycin sulfate used as the positive control as an organic seed disinfectant. There are many reports on the antimicrobial activities of the essential oils and extracts of *Thymus* and *Satureja* species tested against plant pathogens.[5,6,8,23,28,36] However, evaluating plant extracts and oils as seed treatments is a new research area, so there is currently little data on their efficacy. A substantial reduction of infection can be achieved by chemical treatment of the seed.[37,38] On the other hand, chemicals cause health hazards and environmental pollution. For this reason, alternative means of seed treatment have drawn the attention of plant pathologists worldwide. Hence, this study was carried out to evaluate the effectiveness of the essential oil of *S. spicigera*, thymol and carvacrol as organic seed disinfectants or for controlling seed-borne plant pathogenic bacteria.

In conclusion, the present results show that carvacrol, thymol and hydrodistillated oils of *S. spicigera* and *T. fallax*, as well as the hexane, choloroform and acetone extracts of *S. spicigera*, may be

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Table 12. The number of tomato, pepper and lettuce seeds infected with pathogenic bacteria treated with *S. spicigera* essential oil, carvacrol and thymol

		Number of infected seeds*				
Treatments	Concentration (mg mL^{-1})	Cmm (tomato)	Pst (tomato)	Xav (tomato)	Xav (pepper)	Xcvi (lettuce)
The oil	100	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
	40	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
	20	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
	10	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
	4	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
	2	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
Thymol	100	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
	40	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	7.33 ± 1.1^{b}	0.00 ± 0.0^{a}
	20	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	8.00 ± 0.7^{b}	0.00 ± 0.0^{a}
	10	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	14.00 ± 0.7^{c}	0.00 ± 0.0^{a}
	4	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	20.00 ± 0.0^{d}	0.00 ± 0.0^{a}
	2	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	20.00 ± 0.0^{d}	0.00 ± 0.0^{a}
Carvacrol	100	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	2.00 ± 0.7^{a}	0.00 ± 0.0^{a}
	40	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	6.00 ± 1.4^{b}	0.00 ± 0.0^{a}
	20	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	8.67 ± 0.5^{c}	0.00 ± 0.0^{a}
	10	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	20.00 ± 0.0^{d}	0.00 ± 0.0^{a}
	4	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	20.00 ± 0.0^{d}	0.00 ± 0.0^{a}
	2	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	20.00 ± 0.0^{d}	0.00 ± 0.0^{a}
Streptomycin sulfate	0.5	10.66 ± 2.2^{b}	15.00 ± 1.9^{b}	20.00 ± 0.0^{b}	20.00 ± 0.0^{b}	0.00 ± 0.0^{a}
Hot water	–	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
10% DMSO (control)	–	20.00 ± 0.0^{c}	20.00 ± 0.0^{c}	20.00 ± 0.0^{b}	20.00 ± 0.0^{b}	20.00 ± 0.0^{b}

* Values are given as the mean ± SE of three experiments. Different letters in a column indicate statistically significant differences according to the Duncan test $\alpha = 0.05$). Cmm, *C. michiganensis* subsp. *michiganensis*; Pst, *P. syringae* pv. *tomato*; Xav, *X. axonopodis* pv. *vesicatoria*; Xavi, *X. axonopodis* pv. *vitians*.

used as potential antibacterial agents against phytopathogenic bacteria.

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