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Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*

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Abstract

The chemical composition of essential oils isolated from the aerial parts by hydrodistillation of Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum* were analyzed by GC–MS. The oils contain similar major components. The major components of *T. aucheranum* oil were 1,8-cineole (23.8%), camphor (11.6%), terpinen-4-ol (7.2%), α-terpineol (6.5%), borneol (3.8%), (*E*)-thujone (3.2%), *epi*-α-cadinol (3.1%), and artemisia ketone (3.0%). Camphor (17.9%), 1,8-cineole (16.6%) and borneol (15.4%) were found to be predominant constituents in the oil of *T. chiliophyllum*. It is interesting to find that ester derivatives of dihydro-α-cyclogeranic acid (2,2,6-trimethylcyclohexylcarboxylate), dihydro-α-cyclogeranyl hexanoate (10.1%), dihydro-α-cyclogeranyl pentanoate (3.0%), dihydro-α-cyclogeranyl butanoate (2.1%) and dihydro-α-cyclogeranyl propionate (1.2%) are firstly found as chemotaxonomically important components in *T. chiliophyllum* oil. From these, dihydro-α-cyclogeranyl hexanoate was isolated on silica gel column chromatography and its structure was confirmed by spectroscopic methods. This is the first report on the occurrence of ester derivatives of dihydro-α-cyclogeranic acid in essential oils of *Tanacetum* species. The oils were also characterized to have relatively high amounts of oxygenated monoterpenes. Results of the antifungal testing by microbial growth inhibition assays showed that the oils completely inhibit the growth of 30 phytopathogenic fungi. However, their growth inhibition effects were lower than commercial benomyl. The oils tested for antibacterial activity against 33 bacterial strains showed a considerable antibacterial activity over a wide spectrum. Herbicidal effects of the oils on seed germination of *Amaranthus retroflexus*, *Chenopodium album* and *Rumex crispus* were also determined and the oils completely inhibited the seed germination and seedling growth of the plants.

Keywords: *Tanacetum*; *Tanacetum aucheranum*; *Tanacetum chiliophyllum*; Antimicrobial activity; Herbicidal effect; Dihydro-α-cyclogeranic acid esters

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1. Introduction

In recent years, scientists have focused on the increase of food production needed for the fast expansion of world population. Unfortunately, crop losses are continuing due to insects and plant diseases caused by fungi, bacteria and viruses. Microorganisms have also unfavorable effects on quality, safety and preservation of food. Synthetic chemicals are widely used in the control of plant diseases. However, these chemicals are associated with undesirable effects and toxic residues in the products (Barnard et al., 1997; Isman, 2000). Synthetic pesticides also cause environmental pollution owing to their slow biodegradation (Barnard et al., 1997). In addition, the risk of developing the resistance by microorganisms and the high cost–benefit ratio are other disadvantages of synthetic pesticide use (Brent and Hollomon, 1998; Roy and Dureja, 1998).

The control of plant bacterial diseases remains difficult due to the limited availability of bactericides. Bacterial species may cause infectious diseases in humans and may develop resistance to many antibiotics due to the indiscriminate use of commercial antibiotics (Service, 1995). Antibiotics are sometimes associated with adverse effects including hypersensitivity, allergic reaction and immune-suppression (Mukherjee et al., 2002). Therefore, there has been a growing interest in research concerned with alternative antimicrobial sources, including plant extracts, essential oils and secondary metabolites (Rabenhorst, 1996; Misra and Pavlostathis, 1997; Isman, 2000; Yao and Tian, 2005). Hence, our interest has focused on the analyses and effectiveness of essential oils (Cakir et al., 2004; Kordali et al., 2005a,b).

Weeds are another major problem in world agriculture because they cause losses in crop yields. Therefore, farmers have increased herbicide use. However, intensive use of synthetic herbicides can result in soil and groundwater contamination, and development of weed resistance (Abrahim et al., 2000). Herbicides at high concentrations can also increase the risk of toxic residues in agricultural products. Therefore, there is a need for new herbicides that are relatively less harmful for mammalian health and the environment. Thus, researchers have focused on new potential bioherbicides, having different and selective herbicidal mechanisms in comparison to their synthetic herbicides (Dudai et al., 1999; Duke et al., 2000; Tworkoski, 2002).

The genus *Tanacetum*, which is an important member of the Compositae family, is widespread in Europe and western Asia and consists of about 150–200 species. These species have traditionally been used as a spicy additive for food, in cosmetics and as herbal remedies due to their biologically active compounds (Rohloff et al., 2004). This genus, represented in Turkish flora by 44 species and altogether 59 taxa, is rich in essential oils, bitter substances and sesquiterpene lactones (Davis, 1982; Baytop, 1999). *Tanacetum* species are known in Anatolia as ''pireotu'' and their essential oils are used as repellent against insects (Baytop, 1999; Baser et al., 2001). The chemical composition of Turkish *Tanacetum chiliophyllum* has been reported (Baser et al., 2001), but no study has been reported on the antifungal, antibacterial and herbicidal activities of Turkish *T. chiliophyllum* and *Tanacetum aucheranum*. The objective of this study is to determine the toxicity of the essential oils isolated from the aerial parts of *T. aucheranum* and *T. chiliophyllum* var. *chiliophyllum* against 30 phytopathogenic fungi, 33 bacterial strains of plant, clinic and food origins, and the herbicidal effects against three important weeds (*Amaranthus retroflexus*, *Chenopodium album* and *Rumex crispus*).

2. Material and methods

2.1. Plant materials

The aerial parts of *T. aucheranum* and *T. chiliophyllum* were collected from Palandoken Mountain in Erzurum (northeast Turkey) in July 2005 at the flowering stages and were dried in shade. Voucher specimens (ATA-9793 and ATA-9794, respectively) have been deposited in the Herbarium of Atatürk University, Erzurum (Turkey).

2.2. Isolation procedure

The dried plant samples (500 g) were subjected to direct hydrodistillation (plant material in boiling water) by using a Clevenger apparatus for 4 h. The oils were dried over anhydrous Na_2SO_4. Hydrodistillation of *T. aucheranum* and *T. chiliophyllum* yielded 0.15% and 0.22% (w/w) of essential oils. The yields were based on dry mass of the plant samples.

The oil of *T. chiliophylllum* (500 mg) was subjected to silica gel column chromatography (silica gel 60, 180 g, 70–230 mesh) using a solvent mixture *n*-hexane–ethyl acetate (95:5, 90:10, 85:15, 80:20, 75:25) to isolate one of

the major components of *T. chiliophyllum* essential oils. Fractions were collected in 20 ml volume and then each fraction was controlled on thin layer chromatography (TLC). The spots on TLC were visualized by UV_{254nm}, UV_{366nm}, and iodine vapor and by spraying with 1% vanillin–H_2SO_4. The fractions containing the spots of the same compounds were combined and controlled on GC–MS. The 18. and 19. fractions (90 mg) contained mainly dihydro-α-cyclogeranyl hexanoate, but in low purity (approximately relatively 75%). Then, the compound (20 mg) was further purified on preparative TLC (silica gel 60 precoated plates, F_{254} (Merck), layer thickness 0.25 mm) using *n*-hexane–diethyl ether–methanol mixture (40 ml:10 ml:1.5 ml). Its chemical structure was determined by MS, IR, ^{1}H NMR, ^{13}C NMR and 2D NMR (DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC) spectroscopic methods and by comparing with previously reported spectral data (Fehr and Galindo, 1995; Yamamoto et al., 2003, 2004) as hexyl ester of dihydro-α-cyclogeranic acid. Other minor ester derivatives of dihydro-α-cyclogeranic acid were characterized based on the M^+ peak and their fragmentation patterns in MS spectra and by comparing the MS spectra of dihydro-α-cyclogeranyl hexanoate (Fig. 1) and they have similar mass fragmentation patterns. The MS data of the ester derivatives of dihydro-α-cyclogeranic acid, determined in the oil of *T. chiliophyllum* are given below.

Dihydro-α-cyclogeranyl hexanoate (hexyl 2,2,6-trimethylcyclohexylcarboxylate): molecular formula $C_{16}H_{30}O_2$, MW 254. EIMS 70 eV, *m/z* (rel. int): 254 [M^+] (0.8), 253 [$M^+ - H$] (5), 208 (8), 169 [$C_{10}H_{17}O_2^+$] (15), 168 [$C_{10}H_{16}O_2^+$] (72), 154 (26), 153 [$C_{10}H_{17}O^+$] (100), 124 ($C_9H_{16}^+$] (22), 109 (18), 108 (12), 85 [$C_6H_{13}^+$] (41), 57 [$C_4H_9^+$] (43) (Fig. 1).

Dihydro-α-cyclogeranyl pentanoate (pentyl 2,2,6-trimethylcyclohexylcarboxylate): molecular formula $C_{15}H_{28}O_2$, MW 240. EIMS 70 eV, *m/z* (rel. int): 240 [M^+] (0.5), 239 [$M^+ - H$] (3), 194 (12), 169 [$C_{10}H_{17}O_2^+$] (9), 168 [$C_{10}H_{16}O_2^+$] (51), 154 (12), 153 [$C_{10}H_{17}O^+$] (100), 124 [$C_9H_{16}^+$] (22), 109 (15), 108 (12), 93 (9), 71 [$C_5H_{11}^+$] (53).

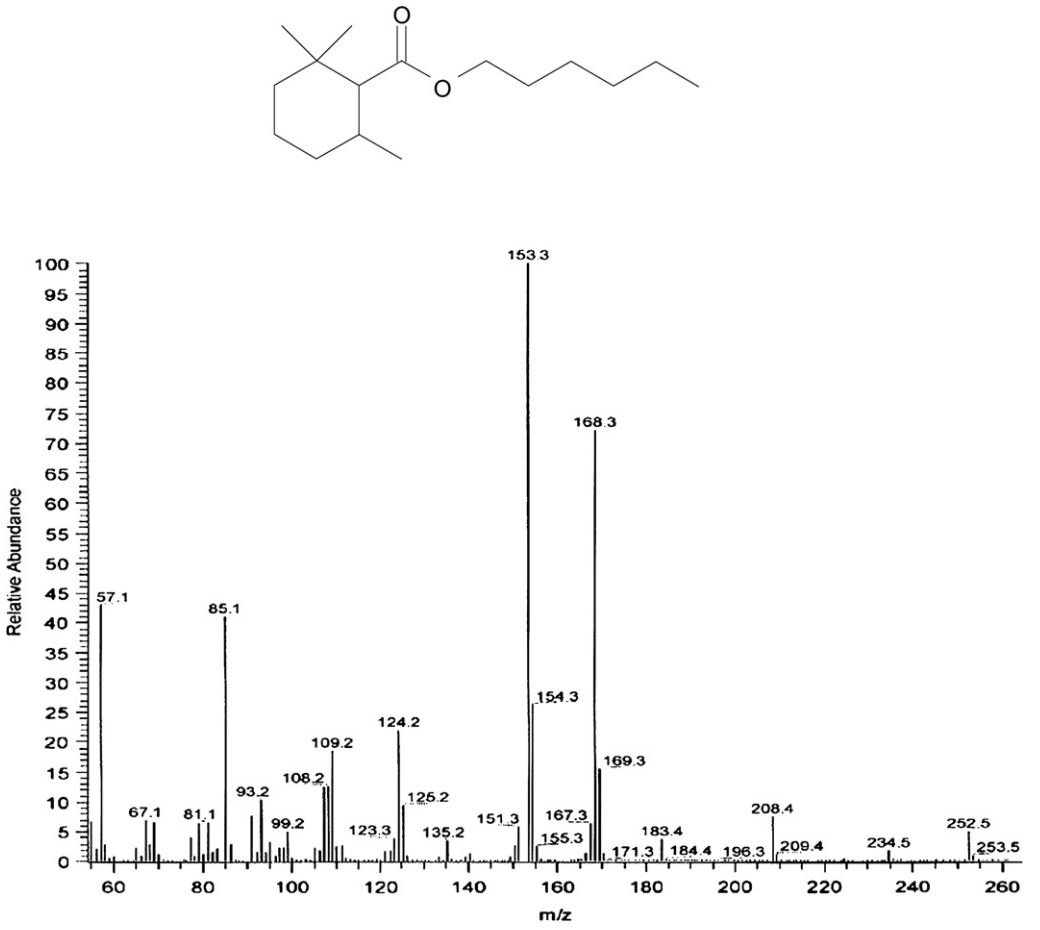

Fig. 1. Chemical structure and mass spectrum of dihydro-α-cyclogeranyl hexanoate detected in the oil of *T. Chiliopyllum.*

Dihydro-α-cyclogeranyl butanoate (butyl 2,2,6-trimethylcyclohexylcarboxylate): molecular formula $C_{14}H_{26}O_2$, MW 226. EIMS 70 eV, *m/z* (rel. int): 226 [M^+] (6), 169 [$C_{10}H_{17}O_2^+$] (9), 168 [$C_{10}H_{16}O_2^+$] (51), 153 [$C_{10}H_{17}O^+$] (100), 152 (28), 124 [$C_9H_{16}^+$] (10), 109 (15), 107 (14), 93 (11), 57 [$C_4H_9^+$] (42).

Dihydro-α-cyclogeranyl propionate (propyl 2,2,6-trimethylcyclohexylcarboxylate): molecular formula $C_{13}H_{24}O_2$, MW 212. EIMS 70 eV, *m/z* (rel. int): 212 [M^+] (4), 210 [$M^+ - 2H$] (5), 168 [$C_{10}H_{16}O_2^+$] (32), 153 [$C_{10}H_{17}O^+$] (100), 152 (22), 124 [$C_9H_{16}^+$] (8), 109 (12), 107 (15), 99 (12).

2.3. Qualitative and quantitative analysis

2.3.1. Gas chromatography–mass spectrometry (GC–MS)

The analysis of the essential oils was performed with a Thermofinnigan Trace GC/Trace DSQ/A1300 (E.I Quadrapole), equipped with SGE-BPX5 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 °C and 290 °C, respectively. The oven temperature was programmed from 50 °C 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 by peak area normalization. The compounds identified in the oils are listed in Table 1.

The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on the SGE-BPX5 capillary column, and by matching their mass spectra peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data (Jennings and Shibamoto, 1980; Adams, 1995, 2007).

2.3.2. Nuclear magnetic resonance (NMR)

^1H and ^{13}C NMR spectra were recorded on a 400 (100)-MHz *Varian spectrometer*, δ in parts per million (ppm), using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard.

2.4. Antifungal activity assays

Thirty agricultural pathogenic fungi were obtained from the culture collection at Atatürk University (Faculty of Agriculture, Department of Plant Protection). Cultures of each of the fungi were maintained on potato dextrose agar (PDA) and were stored at +4 °C. The fungal species used in the experiments are shown in Table 2.

Antifungal activity was studied by using a contact assay (*in vitro*), which produces hyphal growth inhibition (Kordali et al., 2005a). Briefly, potato dextrose agar (PDA) plates were prepared using 9 cm diameter glass Petri dishes. The oil was dissolved in ethanol (1/1; v/v) and suitable amounts (30 μl/Petri dish or 1.5 ml/l concentration) were added to each of the PDA plates containing 20 ml of agar at 50 °C. A disc (5 mm diameter) of the fungal species was cut from 1-week-old cultures on PDA plates and then the mycelia surface of the disc was placed upside down on the centre of a dish with fungal species in contact with growth medium on the dish. Then, the plates were incubated in the dark at 22 ± 2 °C. The extension diameter (mm) of hyphae from centers to the sides of dishes was measured at 24 h intervals for 6 days. Mean of growth measurements were calculated from three replicates of each of the fungal species. PDA plates containing ethanol (30 μl/Petri dish), without essential oil solutions, were used as negative control. In addition, PDA plates treated with benomyl (12.0 mg/Petri dish or 600 mg/l concentration) were used as positive control.

The percentage of growth inhibition by treatment was calculated using the following equation:

$$\%\text{Inhibition} = \frac{C - T}{C} 100$$

where C is the average of four replicates of hyphal extension (mm) of controls and T is the average of four replicates of hyphal extension (mm) of plates treated with essential oil solutions.

Table 1
Chemical composition of *T. aucheranum* and *T. chiliophyllum* essential oils

Constituents	RI[a]	*T. aucheranum* (%)	*T. chiliophyllum* (%)	Identification methods[b]
Tricyclene	905	tr	–	GC, MS
α-Pinene	917	0.5	0.1	GC, MS
Sabinene	972	tr	–	GC, MS
β-Pinene	978	1.0	0.1	GC, MS
3-Carene	1014	0.1	–	GC, MS
α-Terpinene	1026	0.4	–	GC, MS
p-Cymene	1038	1.3	0.6	GC, MS
1,8-Cineole	1045	23.8	16.6	GC, MS
γ-Terpinene	1070	–	0.3	GC, MS
Artemisia ketone	1071	3.0	–	MS
(*Z*)-Linalol oxide (furanoid)	1084	0.2	0.2	GC, MS
Terpinolene	1094	0.7	–	GC, MS
(*E*)-Linalol oxide (furanoid)	1099	–	0.2	GC, MS
p-Cymenene	1103	0.3	–	MS
Linalool	1111	1.9	0.4	GC, MS
(*Z*)-Thujone	1118	0.5	1.1	GC, MS
(*E*)-Thujone	1124	3.2	–	MS
α-Fenchol	1128	–	0.2	GC, MS
(*Z*)-*p*-Menth-2-en-1-ol	1132	1.3	0.5	MS
α-Campholenal	1134	tr	tr	MS
Iso-3-Thujanol	1142	2.1	–	GC, MS
(*E*)-Pinocarveol	1145	–	1.2	MS
(*E*)-*p*-Menth-2-en-1-ol	1146	0.9	–	MS
Camphor	1151	11.6	17.9	GC, MS
Pinocarvone	1162	1.2	2.1	MS
Borneol	1168	3.8	15.4	GC, MS
Terpinen-4-ol	1173	7.2	tr	GC, MS
Napthalene	1178	1.2	0.8	GC, MS
α-Terpineol	1184	6.5	2.0	GC, MS
(*E*)-Piperitol	1193	0.6	0.4	MS
(*E*)-Carveol	1200	0.3	0.5	GC, MS
(*E*)-Chrysanthenyl acetate	1203	1.0	–	MS
Isobornyl formate	1206	–	0.4	MS
Cuminaldehyde	1215	tr	–	GC, MS
Carvone	1218	–	0.5	GC, MS
Piperitone	1221	0.2	–	MS
Bornyl acetate	1234	0.3	1.8	GC, MS
Thymol	1241	0.2	0.4	GC, MS
Carvacrol	1245	0.5	0.2	GC, MS
(*E*)-Dihydro-α-terpinyl acetate	1249	0.1	0.2	MS
(*Z*)-Pinocarvyl acetate	1258	0.1	0.2	GC, MS
Isodihydrocarveol acetate	1259	–	0.3	MS
Eugenol	1271	0.1	0.1	GC, MS
α-Copaene	1276	0.2	tr	GC, MS
Isobornyl propionate	1278	–	0.3	MS
(*E*)-β-Damascenone	1280	–	tr	GC, MS
(*Z*)-β-Damascenone	1281	0.1	–	MS
β-Cubebene	1282	0.1	0.2	GC, MS
Isolongifolene	1284	tr	–	GC, MS
(*E*)-Jasmone	1290	–	0.2	GC, MS
Nepetalactone (4aα, 7β, 7aα)	1289	0.4	–	MS
Methyl eugenol	1292	0.4	–	MS
Bornyl butanoate[c]	1295	–	0.3	MS
β-Caryophylene	1296	0.2	–	GC, MS
(*Z*)-β-Farnesene	1309	0.1	0.1	MS
Dihydro-α-cyclogeranyl propanoate[c]	1311	–	1.2	MS

(*continued on next page*)

Table 1 (*continued*)

Constituents	RI[a]	*T. aucheranum* (%)	*T. chiliophyllum* (%)	Identification methods[b]
allo-Aromadendrene	1312	0.5	–	GC, MS
γ-Muurolene	1317	0.2	–	MS
Germacrene-D	1321	0.7	0.2	MS
α-Selinene	1323	–	0.4	MS
Isobornyl isovalerate	1330	–	0.3	MS
δ-Cadinene	1333	1.6	–	MS
Isobornyl 2-methyl butyrate	1334	–	0.6	MS
(*Z*)-Calamenene	1336	tr	–	MS
α-Cadinene	1339	0.2	–	MS
α-Calacorene	1343	0.1	–	MS
Dihydro-α-cyclogeranyl butanoate[c]	1346	–	2.1	MS
Spathulenol	1357	1.0	2.0	MS
Caryophyllene oxide	1359	1.5	–	GC, MS
Dihydro-α-cyclogeranyl pentanoate[c]	1364	–	3.0	MS
Bornyl tiglate	1369	–	1.0	MS
Isoamyl nerolate	1376	–	0.3	MS
epi-α-Cadinol	1386	3.1	–	MS
β-Eudesmol	1392	2.1	2.6	MS
Junicedranone	1403	–	1.4	MS
Dihydro-α-cyclogeranyl hexanoate	1408	–	10.1	MS, NMR
(*E*,*E*)-Farnesol	1423	–	0.2	GC, MS
Farnesyl acetone[c]	1444	0.1	0.2	MS
Grouped components (%)				
Monoterpene hydrocarbons		4.0	1.1	
Oxygenated monoterpenes		70.9	82.0	
Sesquiterpene hydrocarbons		4.0	0.9	
Oxygenated sesquiterpenes		7.7	6.2	
Others		2.1	1.2	
Total identified		88.7	91.4	

tr: Trace (less than 0.1%).

[a] Retention index relative to *n*-alkanes on SGE-BPX5 capillary column.

[b] Methods: GC, identification 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 (Jennings and Shibamoto, 1980; Adams, 1995, 2007).

[c] The exact isomer was not identified.

2.5. Antibacterial activity assays

The essential oils of *T. aucheranum* and *T. chiliophyllum* species were individually tested against a total of 33 bacterial strains (plant, clinic and food origins) listed in Table 3. Clinical microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine and the Plant Diagnostic Laboratory, Faculty of Agriculture, Atatürk University, Erzurum, Turkey. Phytopathogenic bacterial strains have been isolated from some fruits and vegetables displaying typical bacterial disease symptoms on host plants. It was observed that hypersensitivity test results of some phytopathogenic bacterial strains were positive on tobacco plants (*Nicotiana tabacum* L. var. Samsun) (Table 3). Bacterial cultures were preserved in Luria Broth and 15% glycerol solution at −80 °C prior to use.

Antibacterial activity assays of the oil and penicillin were carried out by the disc diffusion method on Triptic Soy Agar (TSA) medium. Bacterial suspension (50 μl) adjusted to 10^8 CFU/ml final cell concentration was poured into Petri dishes flasks (9 cm) containing 25 ml sterile (TSA) and then was spread by a sterile swab. The oils were sterilized by filtering through 0.45 μm Millipore filters. Sterilized discs (5 mm) were impregnated with 10 μl of the oils and placed on the center of the inoculated agar. Penicillin was used as a positive control by adding 1 mg of penicillin to 1 ml sterilized and distilled water and the sterilized disc was soaked with 10 μl of this solution, corresponding to 10 μg/disk. Bacterial cultures of plant origin were incubated for 3 days at 27 ± 2 °C, whereas the bacterial cultures

Table 2
Antifungal activities of essential oils of *T. aucheranum* and *T. chiliophyllum*

Fungal species	*T. aucheranum* (30 µl/Petri dish)		*T. chiliophyllum* (30 µl/Petri dish)		Benomyl (12 mg/Petri dish)		Control
	Growth[a] (mm)	Inhibition (%)	Growth[a] (mm)	Inhibition (%)	Growth[a] (mm)	Inhibition (%)	Growth[a] (mm)
Alternaria alternata	13.0 ± 1.5	58.1***	9.2 ± 1.0	70.3***	14.7 ± 1.2	52.6***	31.0 ± 3.5
Alternaria solani	12.2 ± 1.2	42.2***	8.7 ± 0.9	58.8***	12.9 ± 1.2	38.9***	21.1 ± 2.7
Botrytis sp.	12.2 ± 2.2	78.4***	9.6 ± 1.1	83.0***	5.0 ± 0.0	91.2***	56.6 ± 7.6
Colletotrichum sp.	12.2 ± 1.7	49.0***	7.1 ± 0.7	70.3***	13.7 ± 1.4	42.7*	23.9 ± 3.8
Drechslera sp.	6.9 ± 0.6	78.0***	5.6 ± 0.2	82.1***	9.7 ± 0.8	69.0***	31.3 ± 2.8
Fusarium acuminatum	11.7 ± 1.3	62.5***	6.9 ± 0.5	77.9***	5.0 ± 0.0	84.0***	31.2 ± 3.9
Fusarium chlamydosporum	8.6 ± 0.9	66.7***	6.4 ± 0.4	75.2***	5.0 ± 0.0	80.6***	25.8 ± 3.5
Fusarium culmorum	10.2 ± 1.0	67.0***	6.8 ± 0.5	78.0***	5.0 ± 0.0	83.8***	30.9 ± 5.0
Fusarium equiseti	5.8 ± 0.4	65.5***	5.5 ± 1.2	67.3***	5.0 ± 0.0	70.2***	16.8 ± 2.1
Fusarium graminearum	10.2 ± 1.2	73.2***	7.4 ± 0.7	80.6***	5.0 ± 0.0	86.9***	38.1 ± 5.1
Fusarium incarnatum	10.2 ± 1.1	35.0	7.1 ± 0.5	54.8***	5.0 ± 0.0	68.2***	15.7 ± 1.7
Fusarium nivale	5.0 ± 0.0	83.1***	5.0 ± 0.0	83.1***	5.0 ± 0.0	83.1***	29.6 ± 4.3
Fusarium oxysporum	12.9 ± 1.4	53.4***	8.0 ± 0.8	71.1***	5.0 ± 0.0	81.9***	27.7 ± 3.7
Fusarium proliferatum	12.3 ± 1.3	64.2***	8.5 ± 0.7	75.3***	5.0 ± 0.0	85.5***	34.4 ± 4.6
Fusarium sambucinum	10.4 ± 1.1	77.5***	7.2 ± 0.5	84.4***	5.0 ± 0.0	89.2***	46.2 ± 6.1
Fusarium scirpi	8.1 ± 0.8	50.9***	6.1 ± 0.4	63.0***	5.0 ± 0.0	69.7***	16.5 ± 5.3
Fusarium semitectum	6.2 ± 0.5	84.1***	5.3 ± 0.2	86.4***	5.0 ± 0.0	87.2***	39.1 ± 5.3
Fusarium solani	15.3 ± 1.7	34.3	13.9 ± 1.6	40.3	5.0 ± 0.0	78.5***	23.3 ± 2.7
Fusarium tabacinum	7.1 ± 0.5	56.7***	5.2 ± 0.1	68.3***	5.0 ± 0.0	69.5***	16.4 ± 2.0
Fusarium verticillioides	10.0 ± 1.1	58.3***	7.7 ± 0.7	67.9***	5.0 ± 0.0	79.2***	24.0 ± 2.9
Nigrospora sp.	7.2 ± 0.7	33.9***	5.2 ± 9.0	52.3***	5.0 ± 0.0	54.1***	10.9 ± 1.0
Phoma sp.	8.2 ± 0.7	57.1***	5.2 ± 0.1	72.8***	5.0 ± 0.0	73.8***	19.1 ± 2.4
Pythium ultimum	7.3 ± 0.7	90.5***	5.0 ± 0.0	93.5***	19.1 ± 3.0	75.2***	77.1 ± 5.5
Phytophthora capsici	14.6 ± 1.4	53.5***	10.8 ± 1.1	65.6***	5.2 ± 0.1	83.4***	31.4 ± 3.3
Rhizoctonia solani	5.3 ± 0.2	90.7***	5.0 ± 0.0	91.2***	5.0 ± 0.0	91.2***	56.9 ± 7.4
Sclerotinia minor	5.4 ± 0.2	85.9***	5.7 ± 0.2	85.2***	5.0 ± 0.0	87.0***	38.4 ± 8.2
Sclerotinia sclerotiorum	19.4 ± 5.0	68.8***	5.3 ± 0.2	91.5***	5.0 ± 0.0	92.0***	62.2 ± 8.3
Sclerotinia sp.	5.0 ± 0.0	87.0***	5.0 ± 0.0	87.0***	29.8 ± 3.9	22.4	38.4 ± 6.1
Trichothecium sp.	6.2 ± 0.4	82.5***	6.0 ± 0.3	83.1***	5.0 ± 0.0	85.9***	35.4 ± 5.4
Verticillium dahliae	6.0 ± 0.3	54.5***	5.4 ± 0.2	59.1***	5.0 ± 0.0	62.1***	13.2 ± 1.4

*: Significant at $p < 0.05$; ***: significant at $p < 0.001$.
[a] The growth of fungal species are given as mean ± SE of three replicates.

of clinic and food origins were incubated at 35 ± 2 °C for the same period. At the end of 3 days, the diameter (mm) of the inhibition zones was measured around the disks. All tests were made in triplicate.

The minimal inhibition concentration (MIC) values were determined for the bacterial strains to the essential oils of *T. aucheranum* and *T. chiliophyllum* by using the modified agar-well diffusion method (Okeke et al., 2001). In the agar-well diffusion technique, a twofold serial dilution of the oils was prepared by diluting methanol to achieve a decreasing concentration range of 1000–6.80 µl/ml. A volume of 100 µl of suspension containing 10^8 CFU/ml of bacteria was spread on Triptic Soy Broth (TSB) plates and disks (6 mm in diameter) impregnated with 10 µl of essential oils were placed on the inoculated agar. Methanol was used as a negative control. All test plates were incubated at 25 °C for bacteria of plant origin and 37 °C for bacteria of clinic and food origin for 24 h. The least concentration of the oils showing a clear zone of inhibition was taken as the MIC. Each assay in this experiment was repeated three times.

2.6. Seed germination and seedling growth experiments

The seeds of *A. retroflexus*, *C. album* and *R. crispus* were collected from the campus of Atatürk University in Erzurum (Turkey) in September–October 2005. To avoid possible inhibition caused by toxins in fungi or bacteria, the seeds were sterilized with 15% sodium hypochlorite for 20 min. They were then rinsed with abundant distilled water. Empty and undeveloped seeds were discarded by floating in tap water and the remaining seeds were air-dried. Two filter papers were placed on the bottom of each Petri dish (9 cm × 1.5 cm deep) and 50 seeds of the respective plant species were placed on the filter papers (Tworkoski, 2002). Then, 10 ml of distilled water was added to each Petri dish.

Table 3
Antibacterial activities of the essential oils of *T. aucheranum* and *T. chiliophyllum*

Bacterial strains	*T. aucheranum*		*T. chiliophyllum*		Penicillin (10 μg/disk)
	Zone (mm)[a]	MIC (μl/ml)	Zone (mm)[a]	MIC (μl/ml)	
Plant pathogenic bacteria					
Gram-positive					
*Clavibacter michiganense**	21.0	166.7	16.8	166.7	40
Gram-negative					
*Agrobacterium tumefacians**	16.8	166.7	12.3	166.7	17
*Erwinia amylovora**	–	–	–	–	31
*Erwinia caratovora**	17.7	55.4	16.3	55.4	48
*Erwinia chrysanthemi**	15.3	500.0	12.5	500.0	22
*Erwinia rhapontici**	17.8	500.0	19.8	500.0	17
*Pseudomonas chlororaphis**	13.5	166.7	13.3	500.0	25
*Pseudomonas cichorii**	–	–	–	–	20
Pseudomonas syringae pv. *syringae**	7.8	500.0	7.5	500.0	29
Xanthomonas axanopodis pv. *malvecearum**	18.8	55.4	19.3	166.7	13
Xanthomonas axanopodis pv. *vesicatoria**	–	–	–	–	–
Xanthomonas hortorum pv. *pelargonii**	–	–	–	–	23
Clinic and food origins					
Gram-positive					
Bacillus coagulans	11.8	166.7	10.2	166.7	40
Bacillus subtilis ATCC 6633	–	–	–	–	26
Citrobacter freundii	9.8	166.7	8.8	166.7	21
Enterococcus fecalis ATCC 29122	9.0	1000.0	–	–	24
Staphylococcus aerous ATCC 29213	9.5	1000.0	11.5	1000.0	21
Streptococcus pyogenes ATCC 176	–	–	–	–	42
Gram-negative					
Acinetobacter johnsonii	11.0	166.7	14.3	166.7	23
Acinetobacter calcoacetius	12.7	166.7	12.5	54.4	25
Enterobacter intermedius	10.7	166.7	10.2	54.4	26
Escherichia coli	9.8	166.7	8.8	500.0	10
Hafnia alvei	9.2	55.4	11.3	500.0	20
Kocuria rosea	14.5	166.7	12.5	55.4	47
Leclercia adecarboxylata	–	–	–	–	27
Neisseria subflava	12.0	55.4	11.3	500.0	50
Pseudomonas aeruginosa ATCC 27859	19.8	166.7	16.5	500.0	29
Pseudomonas aeruginosa ATCC 9027	11.0	1000.0	12.0	1000.0	39
Salmonella enteritidis ATCC 13076	13.3	500.0	19.8	500.0	8
Serratia grimesii	10.3	166.7	11.8	500.0	32
Vibrio hollisae	–	–	–	–	20
Klebsiella trevisanii	9.3	500.0	8.2	166.7	15

–: Not active, *: HR (Hypersensitivity Reactions) test results were positive on *Nicotiana tabacum* L. var. Samsun.
[a] Zones are mean diameter of three replicates.

Lids of Petri dishes were lined with a piece of Whatman no. 1 filter paper held in place with a transparent tape. The oils were applied to this paper at 30 μl/Petri dish by dripping them from the micropipette and impregnating the filter paper. The Petri dishes were closed and sealed with adhesive tape to prevent the volatile oils from escaping. The closed Petri dishes were kept at 23 ± 2 °C on a laboratory bench supplied with 12 h of fluorescent light and humidity of 80% (Tworkoski, 2002). After 15 days, the number of germinated seeds was counted and seedling lengths were measured. The assays were arranged in a completely randomized design with three replications including controls.

2.7. Statistical analysis

Data of seed germination, seedling growth and antifungal activity assays were subjected to one-way analysis of variance (ANOVA), using the SPSS 10.0 software package. Differences between means were tested through LSD and values of $p < 0.05$, 0.01 and 0.001 were considered significantly different.

3. Results and discussion

3.1. Essential oil composition

Hydrodistilled essential oils from aerial parts of *T. aucheranum* and *T. chiliophyllum* are analyzed by GC–MS. The compounds identified in the oils and their relative amounts were given in Table 1. This table shows that *T. aucheranum* and *T. chiliophyllum* oils contain similar major components, which are 1,8-cineole (23.8% and 16.6%), camphor (11.6 and 17.9%), borneol (3.8 and 15.4%), α-terpineol (6.5 and 2.0%) and β-eudesmol (2.1 and 2.6%, respectively). The oils of *T. aucheranum* and *T. chiliophyllum* are also dominated by oxygenated monoterpenes (70.8% and 81.9%, respectively). However, terpinen-4-ol, the third major component of *T. aucheranum* oil with 7.2% was found in trace amounts in *T. chiliophyllum* oil. Furthermore, (*E*)-thujone, iso-3-thujanol, artemisia ketone and *epi*-α-cadinol were relatively abundant in the oil of *T. aucheranum*, but were not detected in the oil of *T. chiliophyllum*. It is interesting to find that ester derivatives of dihydro-α-cyclogeranic acid (2,2,6-trimethylcyclohexylcarboxylate), dihydro-α-cyclogeranyl *n*-hexanoate (10.1%), dihydro-α-cyclogeranyl pentanoate (3.0%), dihydro-α-cyclogeranyl butanoate (2.1%) and dihydro-α-cyclogeranyl propionate (1.2%) are firstly determined as chemotaxonomically important components in *T. chiliophyllum* oil. From these, dihydro-α-cyclogeranyl hexanoate was isolated using silica gel column chromatography and its structure was determined by IR, ^{1}H NMR, ^{13}C NMR and 2D NMR methods (DEPT, ^{1}H–^{13}C COSY, ^{1}H–^{1}H COSY and HMBC) and by comparing previously reported data of dihydro-α-cyclogeranic acid (Fehr and Galindo, 1995; Yamamoto et al., 2003, 2004).

Previously, thujane and camphene derivatives (particularly thujones and camphor), together with 1,8-cineole, have been reported to be major components of many species of the *Tanacetum* genus (Baser et al., 2001; Okeke et al., 2001; El-Shazly et al., 2002; Rohloff et al., 2004; Judzentiene and Mockute, 2005; Ozer et al., 2006). Similarly, as shown in Table 1, *T. aucheranum* and *T. chiliophyllum* oils contain a relatively high amount of camphor and 1,8-cineole. Chemical composition of water-distilled essential oil isolated from the flowers of Turkish origin *T. chiliophyllum* has been reported recently (Baser et al., 2001). Compared with the oil of *T. chiliophyllum* in the previous research (Baser et al., 2001), we found considerable differences in terms of chemical composition of the oil isolated from the aerial parts of *T. chiliophyllum* in the present study (Table 1). For instance, although (*Z*)-chrysanthenyl acetate (16.3%) was the second major component in the previous study (Baser et al., 2001), this component was not detected in the oil of *T. chiliophyllum* in the present study. Furthermore, 1,8-cineole (16.6%) and borneol (15.4%) were found at relatively high amounts in the oil of *T. chiliophyllum* in the present study (Table 1), while these components were in much lower amounts (1.5% and 2.1%, respectively) in the previous study (Baser et al., 2001). In addition to these differences, dihydro-α-geranic acid ester derivatives found in relatively high amounts in the present study were not detected in the oil of *T. chiliophyllum* oil reported in the previous research (Baser et al., 2001). These differences can be attributed to the genotypic variation, climatic conditions and different plant organs.

3.2. Antifungal activity of the essential oils

The essential oils isolated from the aerial parts of two *Tanacetum* species were tested for antifungal activity against agriculturally important fungal species and their fungitoxic effects were compared with the commercial antifungal reagent benomyl. The results of antifungal activity assays showed that the oils and benomyl significantly reduced the growth of the fungal species over a very broad spectrum (Table 2). However, the oils were significantly not active against *Fusarium solani*. Inhibitory effects of the oils on the growth of fungal species were lower compared to benomyl. As seen from Table 2, the oils exhibited similar inhibition effects on the growth of tested fungi. In general, there was a correlation between the antifungal activity and percentage of some major components. Table 1 indicated that *T. aucheranum* and *T. chiliopyllum* oils contain similar major components and a high proportion of oxygenated monoterpenes, which may account for their similar antifungal properties.

A previous study of chemical composition and antifungal properties of shoot oil of *Tanacetum annuum* reported that contains sabinene (22.3%) and camphor (13.2%) as predominant constituents (Greche et al., 2000). The oil exhibited the strongest fungitoxicity, completely inhibiting the mycelial growth of *Botrytis cinerea*, *Alternaria solani* and *Verticillium dahliae* at 5000 ppm doses (Greche et al., 2000). Similarly, the oils of *T. aucheranum* and *T. chiliophyllum* inhibited the mycelial growth of these fungal species in the present study. In general, the antifungal activity of essential oils is attributed to their major components. Previous reports showed that camphor and 1,8-cineole

exhibited weak antifungal activity (Pitarokili et al., 2003; Kordali et al., 2005a, 2007; Oxenham et al., 2005). However, *T. aucheranum* and *T. chiliophyllum* oils, which contain mainly 1,8-cineole and camphor had potent antifungal activity over a very wide spectrum, so their antifungal activity may not be attributed to these components. Previous research demonstrated that some oxygenated monoterpenes such as borneol, terpinene-4-ol, α-terpineol, menthol and essential oils containing high proportions of oxygenated monoterpenes have broad spectrum antifungal activity (Pattnaik et al., 1997; Edris and Farrag, 2003; Kordali et al., 2005a,b, 2007). Thus, the antifungal activity of the oils in the present study can be attributed to their high proportions of these oxygenated monoterpenes. However, other major or trace component(s) in the *Tanacetum* oils (Table 1) could give rise to the antifungal activity of the oils. There are also possible synergistic and antagonistic interactions among the components.

3.3. Antibacterial activity of the essential oils

Antibacterial activities of the oils were studied against 33 bacterial strains (Table 3). The oils inhibited the growth of bacterial strains producing a zone diameter of inhibition from 8.2 to 21.0 mm, depending on susceptibility of the tested bacteria. However, the inhibition zones were lower than those of penicillin, which showed wide inhibition zones at a very low concentration. As seen in Table 3, in general, the oils inhibited the growth of similar bacterial strains, which may be attributed to their similar chemical compositions and major components. On the other hand, it is seen from Table 3 that *T. aucheranum* oil was more effective against some bacterial strains than *T. chiliopyllum* oil. For instance, *T. aucheranum* oil inhibited the growth of *Enterococcus fecalis* ATCC 29122, whereas the oil of *T. chiliophyllum* was not active against this bacterial strain.

Although there are numerous reports on the analyses of essential oils from *Tanacetum* species in the literature, some *Tanacetum* oils have been tested against only a limited number of bacteria (Kalodera et al., 1997; El-Shazly et al., 2002). *T. aucheranum* and *T. chiliophyllum* oils showed broad spectrum antibacterial activity against the tested bacterial strains in the present study (Table 3). Oxygenated monoterpenes such as camphor, 1,8-cineole, borneol, α-terpineol and terpinen-4-ol, which were detected in the oils of *T. aucheranum* and *T. chiliophyllum* oils in the present study, have been demonstrated to exhibit antibacterial activity (Pattnaik et al., 1997; Tabanca et al., 2001). We also reported recently the broad spectrum antibacterial activity of essential oils isolated from *Artemisia spicigera* and *Artemisia santonicum* that contain 1,8-cineole, camphor, borneol and terpinene-4-ol as major components (Kordali et al., 2005a). Therefore, the broad spectrum antibacterial activity of *Tanacetum* oils may be attributed to these major components.

3.4. Herbicidal effects of the oils

Herbicidal effects of *T. aucheranum* and *T. chiliophyllum* oils were tested on seed germination and seedling growth of *A. retroflexus*, *C. album* and *R. crispus*, which are important weeds in cultivated areas, and were compared with 2, 4-D isooctylester, a commercial herbicide. The results show that the oils completely inhibited the germination and seedling growth of these three plant species relative to the control (Table 4). The herbicidal effects of some essential oils and their pure constituents against these plant species have been previously reported (Onen et al., 2002; Angelini et al., 2003) and their effects are generally attributed to their major components. For instance, it has been stated that essential oil of *Rosmarinus officinalis* contains 1,8-cineole, α-pinene and camphor as predominant constituents and suppressed the germination of *A. retroflexus* (Angelini et al., 2003). Similarly, the oils of *Ocimum basilicum* and *Salvia officinalis* that contain predominantly camphor and 1,8-cineole were found to be highly phytotoxic on seed germination and seedling growth of *A. retroflexus*, *C. album* and *R. crispus*. Likewise, in the present study, the essential oils of *T. aucheranum* and *T. chiliophylllum*, characterized by the relatively high content of 1,8-cineole, camphor, borneol and α-terpineol, exhibited potent inhibitory effects on seed germination and seedling growth of *A. retroflexus*. These findings suggest that the strong herbicidal effects of the oils on germination and seedling growth of the species tested are probably due to their major components. It has been reported that 1,8-cineole and camphor have strong phytotoxic effects against various plant species (Koitabashi et al., 1997; Abrahim et al., 2000; Vokou et al., 2003; Zunino and Zygadlo, 2004; Nishida et al., 2005; Kordali et al., in press), in agreement with the present results. Furthermore, it has been documented that oxygenated monoterpenes possess relatively high phytotoxic effects in comparison to monoterpene hydrocarbons (Vokou et al., 2003; Kordali et al., in press). The herbicidal effects of *Tanacetum* oils can be attributed to their relatively high content of oxygenated monoterpenes (Table 1). Although the herbicidal

Table 4
Herbicidal effects of the oils on seed germination and seedling growth of *A. retroflexus*, *C. album* and *R. crispus*

Samples	Germination[a] (%)	Seedling growth (mm)[a]	
		Root	Aerial part
A. retroflexus			
Control	76.3 ± 1.9	28.6 ± 0.7	32.4 ± 1.0
T. aucheranum	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
T. chiliophyllum	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
2,4-D	2.0 ± 1.5***	1.9 ± 0.3***	6.3 ± 2.0***
C. album			
Control	57.0 ± 3.6	28.4 ± 0.8	20.7 ± 1.1
T. aucheranum	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
T. chiliophyllum	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
2,4-D	58.0 ± 1.7	5.5 ± 0.3***	12.2 ± 0.4***
R. crispus			
Control	69.0 ± 6.1	20.6 ± 1.1	20.0 ± 0.5
T. aucheranum	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
T. chiliophyllum	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
2,4-D	77.3 ± 3.5	10.8 ± 0.6**	11.1 ± 0.3**

: Statistically significant difference at $p < 0.01$; *: statistically significant difference at $p < 0.001$.
[a] Mean ± SE of three replicates, each containing with 50 seeds.

mechanism of the oils was not investigated in the present study, it is well known that monoterpenes in the essential oils have phytotoxic effects that may cause anatomical and physiological changes in plant seedlings leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such as mitochondria, possibly due to inhibition of DNA synthesis or disruption of membranes surrounding mitochondria and nuclei (Koitabashi et al., 1997; Zunino and Zygadlo, 2004; Nishida et al., 2005). The phytotoxic effects of the oils may be due to one or more of these factors.

In conclusion, the development of natural antimicrobials and pesticides would help to decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution. In this respect, natural pesticides and herbicides may be effective, selective, biodegradable, and less toxic to environment. *T. aucheranum* and *T. chiliophyllum* oils showed significant broad spectra antifungal activity and strong phytotoxic effects against *A. retroflexus*, *C. album* and *R. crispus*. Based on the present results, these oils could be suggested as alternative pesticides and herbicides. However, further studies will be needed to evaluate the cost, efficacy and safety of these oils.

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