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Determination of the Chemical Composition and Antioxidant Activity of the Essential Oil of *Artemisia dracunculus* and of the Antifungal and Antibacterial Activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* Essential Oils

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The essential oil isolated from Turkish tarragon (*Artemisia dracunculus*) by hydrodistillation was analyzed by GC-MS. Thirty compounds representing 99.5% of total oil were identified. The predominant components in the oil were (*Z*)-anethole (81.0%), (*Z*)-β-ocimene (6.5%), (*E*)-β-ocimene (3.1%), limonene (3.1%), and methyleugenol (1.8%). The antibacterial and antifungal activities of the essential oils isolated from *A. dracunculus*, *Artemisia absinthium*, *Artemisia santonicum*, and *Artemisia spicigera* oils were also evaluated. In general, the oils exhibited potent antifungal activity at a wide spectrum on the growth of agricultural pathogenic fungi. Among the oils, the weakest antifungal activity was shown by the oil of *A. dracunculus*. In many cases, the oils of *A. absinthium*, *A. santonicum*, and *A. spicigera* completely inhibited the growth of some fungal species. As compared with antibacterial activities of all of tested oils, *A. santonicum* and *A. spicigera* oils showed antibacterial activities over a very wide spectrum. However, the essential oils tested showed lower inhibition zones than the inhibition zones of penicillin. In addition, antioxidant and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of tarragon oil were determined, and weak antioxidant and DPPH radical scavenging activities were found in comparison to butylated hydroxytoluene.

KEYWORDS: Compositae; *Artemisia*; essential oil; (*Z*)-anethole; antibacterial; antifungal; antioxidant; DPPH radical scavenging

INTRODUCTION

In recent years, scientists have focused on increasing food production to meet the demands of the rapidly expanding world population. Unfortunately, crop loss remains a problem due to plant diseases caused by insects, plant pathogen fungi, bacteria, and viruses. Microorganisms have also unfavorable effects on the quality, safety, and shelf life of foods. Therefore, the postharvest diseases caused by microorganisms are still a most important problem. Synthetic chemicals are widely used in the control of plant diseases. However, these chemicals are associated with undesirable effects and some toxic residues in the products (*1*, *2*). Synthetic pesticides also cause pollution owing to their slow biodegradation in the environment (*1*). In addition, the risk of the development of resistance by microorganisms and the high cost−benefit ratio are other disadvantages of synthetic pesticide uses (*3*, *4*). Microorganisms causing infectious diseases in humans may develop resistance to many antibiotics due to the indiscriminate use of commercial antibiotics (*5*, *6*). In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, allergic reaction, and immunity suppression (*12*). Therefore, there has been a growing interest in research concerning alternative pesticides and antimicrobial active compounds, including the plant extracts and essential oils that are relatively less damaging to the mammalian health and environment (*2*, *7*−*9*). Hence, our interest focused on the analyses and effectiveness of essential oils (*10*−*12*).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in cells by different means (*13*). Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (*14*). ROS and RNS may cause DNA damage that could lead to mutation. All aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damage. However, these natural antioxidant mechanisms can be inefficient, and hence dietary intake of antioxidant compounds

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becomes important. Free radicals can also cause lipid peroxidation in foods that leads to their deterioration. Although there are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), these compounds are associated with some side effects (*15*). Therefore, research in the determination of natural sources of antioxidants and the antioxidant potential of plants is important.

The genus *Artemisia*, small herbs and shrubs, is one of the largest and most widely distributed genera of the Compositae (or Asteraceae) family (*16*, *17*). Members of this genus have botanical and pharmaceutical interest due to their characteristic scent or taste and are used in the liqueur-making industry (*16*, *18*). There are about 22 species of the *Artemisia* genus in Turkey (*16*, *17*). *A. dracunculus* (Tarragon), known as "tarhun" in Anatolia, is a small shrubby perennial herb. It is cultivated for the use of its aromatic leaves in seasoning, salad, and soup (*16*). *A. absinthium*, known locally as "pelin otu", "aci pelin", "ak pelin", and "büyük pelin", grows naturally in wide regions of Anatolia and has been used as an antipyretic, antiseptic, antihelmintic, tonic, and diuretic and for the treatment of stomachache in Turkish folk medicine (*16*). *A. spicigera*, named locally as "yavşan", is widespread in central and eastern Anatolia, at an altitude between 1000 and 2500 m (*16*, *17*). *A. santonicum*, which is known as "deniz yavşanı" and "kokulu yavşan", grows on sandy places and salted lands in Turkey. This species has been used as an antihelmintic and in the treatment of diabetes (*16*). Its antidiabetic activity has been also recently confirmed (*19*).

Numerous studies have been reported on the analysis of the essential oil compositions and their biological activities from various species of *Artemisia* (*18*, *20*−*28*). Recently, our research group has reported the essential oil compositions, antioxidant activities, and antifungal activities against only 11 phytopathogenic fungi of *A. absinthium*, *A. santonicum*, and *A. spicigera* growing in the Erzurum region of Turkey, but not their antibacterial activities (*11*). However, there is so far no report about the chemical composition of the essential oils and their antifungal, antibacterial, and antioxidant activities of Turkish tarragon (*A. dracunculus*). Thus, the aims of the present study are (a) to investigate the chemical composition of the essential oils isolated from the aerial parts of *A. dracunculus* from Turkey; (b) to assess the antifungal and antibacterial activities of the essential oils isolated from *A. dracunculus*, *A. absinthium*, *A. spicigera*, and *A. santonicum* against a group of fungal species (34 agricultural pathogens) and bacteria (plant, food, and clinic origins); and (c) to determine the antioxidant and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of the essential oil of *A. dracunculus*.

EXPERIMENTAL PROCEDURES

Plant Materials. The aerial parts of *A. dracunculus* (ATA-9783) were purchased at vegetative stage from a local market. *A. santonicum* (ATA-9772), *A. spicigera* (ATA-9773), and *A. absinthium* (ATA-9774)-were collected in the Erzurum region of Turkey in July 2004 at the flowering stages and were dried in the shade. Voucher specimens have been deposited in the herbarium of Atatürk University, Erzurum (Turkey).

Isolation of 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. Hydrodistillation of *A. dracunculus*, *A. absinthium*, *A. santonicum*, and *A. spicigera* yielded 1.00, 0.67, 0.85, and 0.60% (w/w) of essential oils, respectively. The yields were based on dry materials of plant samples.

Table 1. Chemical Composition[a] of *A. dracunculus* Essential Oil

RI[b]	constituent	%	identification method[c]
833	*o*-xylene	0.1	MS
916	α-pinene	0.3	GC, MS
970	sabinene	tr[d]	GC, MS
975	β-pinene	0.1	GC, MS
989	β-myrcene	0.1	GC, MS
992	dehydro-1,8-cineole	tr	MS
1034	limonene	3.1	GC, MS
1040	(*Z*)-β-ocimene	6.5	MS
1050	(*E*)-β-ocimene	3.1	MS
1078	(*Z*)-vertocitral C	0.1	MS
1102	linalool	0.3	GC, MS
1123	*allo*-ocimene	0.5	GC, MS
1135	epoxyocimene	0.1	MS
1162	borneol	tr	GC, MS
1184	(*Z*)-anethole	81.0	GC, MS
1209	carvone	0.1	GC, MS
1214	chavicol	tr	MS
1228	bornyl acetate	0.1	GC, MS
1287	methyleugenol	1.8	MS
1290	β-caryophyllene	tr	GC, MS
1314	γ-decalactone	0.1	MS
1318	α-zingiberene	0.2	MS
1323	(*E*,*E*)-α-farnesene	tr	MS
1331	β-sesquiphellandrene	0.1	MS
1345	*trans*-nerolidol	tr	GC, MS
1354	spathulenol	0.9	GC, MS
1358	gleenol	0.5	MS
1424	4-hydroxycoumarin	0.1	MS
1428	myristic acid	tr	MS
1462	phytol[e]	0.2	GC, MS
1473	nuciferol heptanoate[e]	0.1	MS

[a] Percentages obtained by FID peak area normalization. [b] Retention index relative to *n*-alkanes on SGE-BPX5 capillary column. [c] Methods: GC, identification based on retention times of authentic compounds on SGE-BPX5 capillary column; MS, tentatively identified on the basis of computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data (*29*, *30*). [d] Trace (<0.1%). [e] The exact isomer was not identified.

GC-MS Analysis. The analysis of the essential oil was performed with a Thermofinnigan Trace GC/Trace DSQ/A1300 (EI quadrapole) equipped with an SGE-BPX5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness = 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 to 150 °C at 3 °C/min, then held isothermal for 10 min, and finally raised to 250 °C at 10 °C/min. Diluted samples (1:100, v/v, in methylene chloride) of 1.0 μL were injected manually in the splitless mode. The relative percentage of the oil constituents was expressed as percentages by peak area normalization.

The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on the SGE-BPX5 capillary column and by matching of their mass spectra of peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data (*29*, *30*). (*Z*)-Anethole, α-pinene, β-pinene, limonene, linalool, *allo*-ocimene, borneol, carvone, and spathulenol were purchased from Fluka; sabinene, phytol, β-myrcene, and (*E*)-nerolidol were purchased from Aldrich; bornyl acetate and β-caryophyllene were purchased from Sigma.

Fungal Species and Antifungal Activity Assays. The 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 given in **Table 2**. The identification of the fungal species was confirmed by Microbial Identification System (MIS) (in the Biotechnology Application and Research Center of Atatürk University).

Table 2. Antifungal Activities of the Essential Oils of *Artemisia* Species

	growth[a] (mm)					
fungal species	*A. absinthium*	*A. dracunculus*	*A. santonicum*	*A. spicigera*	benomyl	control
Alternaria alternata	24.8 ± 3.0[b]	9.8 ± 1.3***	14.1 ± 1.8***[b]	11.3 ± 1.5***[b]	14.7 ± 1.2***	31.0 ± 3.5
Alternaria solani	8.2 ± 0.9***	7.1 ± 0.7***	5.0 ± 0.0***	5.0 ± 0.0***	12.9 ± 1.2***	21.1 ± 2.7
Aspergillus sp.	6.9 ± 0.6***	15.5 ± 2.3	12.4 ± 1.7	8.5 ± 1.0***	5.2 ± 0.1***	23.7 ± 4.4
Aspergillus niger	9.6 ± 1.2***	6.7 ± 0.5***	8.0 ± 0.7***	8.1 ± 0.7***	6.4 ± 0.3***	40.8 ± 6.1
Botyrtis sp.	5.1 ± 0.2***	36.7 ± 7.3**	8.8 ± 0.8***	7.6 ± 0.7***	5.0 ± 0.0***	56.6 ± 7.6
Dreshlera sp.	5.0 ± 0.0***	8.3 ± 1.1***	5.0 ± 0.0***	5.4 ± 0.2***	9.7 ± 0.8***	31.3 ± 2.8
Fusarium acuminatum	6.1 ± 0.3***	10.7 ± 1.4***	6.4 ± 0.4***	6.7 ± 0.5***	5.0 ± 0.0***	31.2 ± 3.9
Fusarium chlamydosporum	6.5 ± 0.5***	8.7 ± 1.1***	5.6 ± 0.2***	6.9 ± 0.5***	5.0 ± 0.0***	25.8 ± 3.5
Fusarium culmorum	5.5 ± 0.2***	9.3 ± 1.3***	6.3 ± 0.4***	7.2 ± 0.6***	5.0 ± 0.0***	30.9 ± 5.0
Fusarium equiseti	5.6 ± 0.2***	8.7 ± 1.0**	5.0 ± 0.0***	5.4 ± 0.2***	5.0 ± 0.0***	16.8 ± 2.1
Fusarium graminearum	5.6 ± 0.2***	11.4 ± 1.6***	5.6 ± 0.2***	7.7 ± 0.8***	5.0 ± 0.0***	38.1 ± 5.1
Fusarium incarnatum	5.8 ± 0.3***	8.6 ± 1.0	5.2 ± 0.1***	5.5 ± 0.2***	5.0 ± 0.0***	15.7 ± 1.7
Fusarium nivale	5.0 ± 0.0***	8.5 ± 1.3***	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	29.6 ± 4.3
Fusarium oxysporum	18.0 ± 2.1***[b]	16.6 ± 2.7**	12.8 ± 1.4***[b]	12.7 ± 1.5***[b]	5.0 ± 0.0***	27.7 ± 3.7
Fusarium proliferatum	7.6 ± 0.7***	10.9 ± 1.5***	7.3 ± 0.6***	7.1 ± 0.6***	5.0 ± 0.0***	34.4 ± 4.6
Fusarium sambucinum	28.4 ± 3.8***[b]	15.8 ± 2.6***	9.3 ± 1.0[b] ***	10.1 ± 1.2***[b]	5.0 ± 0.0***	46.2 ± 6.1
Fusarium scirpi	5.0 ± 0.0***	8.9 ± 1.0**	5.6 ± 0.2***	5.9 ± 0.3***	5.0 ± 0.0***	16.5 ± 5.3
Fusarium seminectum	5.3 ± 0.1***	17.8 ± 2.7***	5.3 ± 0.1***	5.2 ± 0.1***	5.0 ± 0.0***	39.1 ± 5.3
Fusarium solani	20.1 ± 2.2[b]	16.3 ± 2.1*	20.9 ± 2.5[b]	17.2 ± 2.2*[b]	5.0 ± 0.0***	23.3 ± 2.7
Fusarium tabacinum	5.1 ± 0.1***	12.3 ± 1.6*	5.1 ± 0.1***	5.4 ± 0.2***	5.0 ± 0.0***	16.4 ± 2.0
Fusarium verticillioides	5.2 ± 0.1***	11.3 ± 1.7***	6.3 ± 0.4***	5.8 ± 0.3***	5.0 ± 0.0***	24.0 ± 2.9
Monilya sp.	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	26.1 ± 3.0
Nigrospora sp.	6.1 ± 0.5***	15.9 ± 1.3*	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	10.9 ± 1.0
Penicillium jensenii	5.8 ± 0.3***[b]	17.7 ± 2.3***	5.0 ± 0.0***[b]	6.0 ± 0.4***[b]	5.2 ± 0.1***	37.9 ± 7.1
Phoma sp.	5.1 ± 0.1***	6.8 ± 0.8***	8.8 ± 0.8***	7.6 ± 0.7***	5.0 ± 0.0***	19.1 ± 2.4
Phythium ultimum	5.2 ± 0.2***	23.4 ± 6.4***	5.2 ± 0.2***	5.2 ± 0.2***	19.7 ± 3.1***	77.1 ± 5.5
Phytophera capsisi	7.9 ± 0.6***	13.0 ± 1.9***	7.7 ± 0.6***	8.3 ± 0.5***	5.2 ± 0.1***	31.4 ± 3.3
Rhizoctonia solani	5.1 ± 0.1***	6.1 ± 0.4***	5.1 ± 0.1***	5.1 ± 0.1***	5.0 ± 0.0***	56.9 ± 7.4
Sclerotinia sclerotiorum	5.0 ± 0.0***[b]	26.8 ± 7.5**	5.0 ± 0.0***[b]	10.1 ± 1.2***[b]	5.0 ± 0.0***	62.2 ± 8.3
Sclerotinia sp.	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	29.8 ± 3.9	38.4 ± 6.1
Trichotecium sp.	5.0 ± 0.0***	12.6 ± 2.0***	6.4 ± 0.4***	5.5 ± 0.2***	5.0 ± 0.0***	35.4 ± 5.4
Verticillium albo-atrum	5.0 ± 0.0***[b]	15.7 ± 2.5	5.0 ± 0.0***[b]	5.0 ± 0.0***[b]	5.0 ± 0.0***	16.2 ± 1.8
Verticillium dahliae	5.5 ± 0.2***	7.1 ± 0.7***	5.4 ± 0.2***	6.3 ± 0.4***	5.0 ± 0.0***	13.2 ± 1.4
Verticillium tenerum	5.0 ± 0.0***[b]	9.3 ± 1.3***	5.0 ± 0.0***[b]	5.0 ± 0.0***[b]	5.5 ± 0.0***	21.4 ± 2.9

[a] Growth of fungal species is given as mean ± standard error of three replicates: *, significant at $p < 0.05$; **, significant at $p < 0.01$; ***, significant at $p < 0.001$ according to control. [b] Not tested; results quoted from our previous research (*11*).

Antifungal activity was studied by using contact assay (in vitro), which produces hyphal growth inhibition (*8*, *9*). Briefly, PDA plates were prepared using 9 cm diameter glass Petri dishes. Twenty microliters of the essential oils were added to each of the PDA plates containing 20 mL of agar. A 5 mm diameter disk of the fungal species was cut from a 1-week-old culture on PDA plates, and then the mycelial surface of the disk was placed upside down on the center of dish. Therefore, the fungal species was in contact with the growth medium on the dish. Then, the plates were incubated in the dark at 22 ± 2 °C. The extension diameter (millimeters) of hyphae from the center to the side of the dish was measured at 24 h intervals for 6 days. Mean growth measurements were calculated from three replicates of each of the fungal species. PDA plates treated with distilled water, without essential oil solutions, were used as negative control. In addition, PDA plates treated with benomyl (12.0 mg/Petri dishes) were used as positive controls (*31*).

Antibacterial Activity Assays. The essential oils of four *Artemisia* species were individually tested against a total of 64 bacterial strains (plant, food, and clinic origins). The list of used bacterial strains is given in **Table 3**. Clinical microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine and Plant Diagnostic Laboratory, Faculty of Agriculture, Atatürk University, Erzurum, Turkey. Phytopathogenic bacterial strains have been isolated from some fruits and vegetables exhibiting typical bacterial disease symptoms on host plants. The identities of the microorganisms were confirmed by the Microbial Identification System (MIS) (in the Biotechnology Application and Research Center of Atatürk University (**Table 3**). It was observed that the hypersensitivity test (HR) results of some phytopathogenic bacterial strains were positive on tobacco plants (*Nicotina tabacum* L. var. Samsun) on the basis of the method previously described (**Table 1**) (*32*). Bacterial cultures were preserved in Luria broth and 15% glycerol solution at −80 °C prior to use.

Antibacterial activity assays of the essential oils and penicillin were carried out according to the disk diffusion method on NA (Difco) medium (*33*). Suspensions (100 μL) of the bacteria were adjusted to 10^8 cfu/mL final cell concentration; 50 μL of bacterial suspension was poured into Petri dish flasks (9 cm) containing 25 mL of sterile NA and then spread by a sterile swab. Amounts of 60, 90, and 120 mg of the essential oils were individually dissolved in 1 mL of methanol, and these solutions were sterilized in 0.45 μm Millipore filters. Sterilized disks (5 mm) were impregnated with 10 μL of these essential oil solutions, corresponding to 600, 900, and 1200 μg/disk, respectively, placed on the inoculated agar. These disks were put in the middle of plates. Penicillin was used as a positive control: 1 mg of penicillin was added into 1 mL of sterilized and distilled water, and then the sterilized disk was soaked with 10 μL of this solution, corresponding to 10 μg/disk. Bacterial cultures of plant origins were incubated for 6 days at 27 ± 2 °C, whereas the bacterial cultures of clinic and food origins were incubated at 35 ± 2 °C for the same period. At the end of 6 days, inhibition zones were measured in diameter (millimeter) around the disks. All of the tests were made in triplicate.

Determination of Antioxidant Activity. The antioxidant activity was determined according to the thiocyanate method (*11*). Briefly, stock essential oil solutions were prepared at 20 mg/mL concentration in ethanol. Required stock solutions were mixed with 2.5 mL of 0.02 M linoleic acid (Fluka) emulsion [contains an equal weight of Tween-20 (Sigma) in pH 7.4 phosphate-buffered saline (Sigma)], and the final volume was adjusted to 5 mL with phosphate-buffered saline (0.02 M, pH 7.4) in a test tube and incubated in darkness at 40 °C. Final concentrations of essential oil were 100 and 200 μg/mL. BHT (Sigma)

Table 3. Antibacterial Activities of the Essential Oils of *A. absinthium*, *A. dracunculus*, *A. santonicum*, and *A. spicigera*[a]

bacterial strain	*A. absinthium* 600–1200 μg/disk	*A. dracunculus* 600–1200 μg/disk	*A. santonicum* 600–1200 μg/disk	*A. spicigera* 600–1200 μg/disk	*Penicillin* 10 μg/disk
plant origin					
Agrobacterium tumefaciens	–[b]	8	8–10	8–10	12
Clavibacter michiganense (Cmm)	–	–	10–11	9–10	40
Curtobacterium flaccumfaciens	9	–	10–11	8–9	60
Erwinia amylovora	–	–	8–9	8–10	31
Erwinia ananas (PD-761)	–	–	10	9–11	30
Erwinia caratovora	–	–	10–11	10	54
Pseudomonas syringae pv. *phaseolicola*	–	–	8	–	27
Pseudomonas syringae glycinea (RK-469)	–	–	7–9	7–8	26
Pseudomonas syringae glycinea (RK-470)	–	10–15	7	–	24
Pseudomonas syringae pv. *maculicola*	7–8	8–9	9–14	8–10	32
Pseudomonas syringae pv. *populans*	–	7	-	-	29
Pseudomonas syringae pv. *syringae* (RK-470)	7	7–8	10	7–8	29
Pseudomonas syringae pv. *tabaci*	–	7	8–9	7–8	40
Pseudomonas syringae pv. *tomato*	–	–	–	–	–
Xanthomonas axanopodas pv. *vesicatoria*	11–12	8–14	11–17	10–17	–
Xanthomonas pelargonii	11–13	10	14–16	11–15	23
food origin					
Bacillus subtilus ATCC 6633	–	–	–	–	26
Brevibacillus brevis	–	8–9	9–10	7–9	40
Brevibacterium casei	–	8–13	9–10	8–11	39
Brevundimonas diminuta	–	8–9	8–9	7–8	50
Burkholderia pyriocinia	–	8–9	8	7–10	9
Chromobacterium violaceum	–	–	8	7–9	21
Chryseobacterium indologenes	14–15	7–11	14	8–9	18
Citrobacter freundii	–	7	8–9	7–9	12
Erwinia chrysanthemi	–	–	8–10	8–11	22
Kocuria rosea	9–12	–	11–13	11–12	57
Kocuria varians	–	7–10	8–10	7–9	23
Leclercia adecarboxylata	–	–	7–8	–	17
Micobacterium saperdae	11–12	-	13–16	11–15	65
Ralstonia pickettii	–	7–9	11–12	12–17	35
Serratia grimesii	–	8	8–10	8–10	13
clinic origin					
Acinetobacter baumannii	–	7–10	8–10	8–9	34
Acinetobacter calcoaceticus	–	10	7–8	10–11	25
Acinetobacter johnsonii	–	9–11	7–8	7–8	30
Acinetobacter radioresistens	–	7–9	8–9	7–10	32
Aerococcus vridans	–	7–8	7–9	7–8	10
Arthrobacter spp.	12–14	–	14–15	12–14	41
Bacillus coagulans	–	8–9	10–12	10–14	50
Bacillus megaterium	–	–	7–8	7–9	–
Bacillus mycoides	7–9	–	10–11	8–10	12
Enterobacter cloaceae	–	–	8–9	–	–
Enterobacter intermedius	–	–	8–9	7–9	26
Enterococcus fecalis ATCC 29122	–	7–8	–	7–9	24
Escherichia coli	–	–	8	8–9	-
Hafnia alvai	–	–	8–9	7–9	18
Klebsiella pneumoniae	–	7–8	8–9	7–10	16
Klebsiella trevisanii	–	–	7–8	7–8	15
Klebsiella planticola	–	–	7–8	7	–
Micrococcus luteus	–	–	–	–	59
Micrococcus lylae	11–15	–	13–16	11–15	56
Neisseria mucosa	–	8	7–8	7	17
Neisseria subflava	–	7–8	7–10	8	13
Proteus vulgaris	–	7–15	10–11	8–9	21
Pseudomonas aeruginosa	9–11	7–8	11–12	9–15	41
Pseudomonas aeruginosa ATCC 9027	–	7	–	7–10	39
Pseudomonas aeruginosa ATCC 27859	11–13	9–13	10–14	8–13	29
Salmonella enteridus ATCC 13076	–	–	7–8	7–8	8
Salmonella typhimurium	–	7–9	9	7–10	36
Staphylococcus aureus ATCC 29213	–	–	8	7–8	21
Staphylococcus hominis	–	–	–	8–9	39
Stenotrophomonas maltopholia	–	10	7–8	8–10	32
Streptococcus pyogenes ATCC 176	–	7	8	–	42
Vibrio alginolyticus	–	7	8–9	8–9	19
Vibrio hollisae	–	–	7	–	16

[a] Inhibition zones are given as minimum and maximum inhibition zones in diameter (mm) around the disks impregnated at 600, 900, and 1200 μg/disk doses. [b] Not active.

was used as positive control (100 μg/mL). The amount of peroxide was determined by measuring the absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation. Low absorbance indicates high antioxidant activity. To eliminate the solvent effect, the same amount of solvent used to prepare the solutions of test samples was added into the control test sample, which contains the

linoleic acid emulsion. Measurements of antioxidant activity were carried out for three sample replications, and values are the average of three replicates.

Determination of DPPH Radical Scavenging Activity. Experiments were carried out as described previously (*11*). Briefly, 0.5 mM DPPH (Fluka) radical solution in methanol was prepared, and then 1 mL of this solution was mixed with 3 mL of the sample solution in ethanol. Final concentrations of essential oils were 100, 200, 400, and 1000 μg/mL. BHT was used as a positive control at 100 μg/mL concentration. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as percent DPPH radical scavenging, which is calculated with the equation

$$\%\ \text{DPPH radical scavenging} = [(\text{control absorbance} - \text{sample absorbance})/\text{control absorbance}] \times 100$$

Control contained 1 mL of DPPH solution and 3 mL of ethanol. The measurements of DPPH radical scavenging activity were carried out for three sample replications, and values are an average of three replicates.

Statistical Analysis. To determine whether there is a statistically significant difference among the obtained results for antifungal and antioxidant activity assays, variance analyses were carried out using SPSS 10.0 software package. Values of $p < 0.05$ were considered to be significantly different.

RESULTS AND DISCUSSION

Essential Oil Composition. The hydrodistillation essential oil composition of Turkish *A. dracunculus* and the relative amounts of the components are shown in **Table 1**. This table shows that the Turkish tarragon contains (*Z*)-anethole (81.0%), (*Z*)-β-ocimene (6.5%), (*E*)-β-ocimene (3.1%), limonene (3.1%), and methyleugenol (1.8%) as major components. Turkish tarragon oil is also rich in aromatic components (83.0%). The essential oil composition of tarragon grown in Cuba (*20*), Albany (*21*, *28*), and Iran (*22*) has been previously reported. In these research papers, it has been shown that tarragon contains some differences in terms of chemical composition depending on climatic conditions and chemotypes. The oil of tarragon of Cuban origin contains elemicin (53.0%) and methyleugenol (17.6%) as major components (*20*). In the present study, the relative amount of methyleugenol was found to be 1.8%, whereas elemicin was not detected. However, the chemical composition of Albany tarragon oil was different from that of typical tarragon oil, consisting of mainly terpinolene (25.4%), (*Z*)-β-ocimene (22.2%), 5-phenyl-1,3-pentadiyne (11.7%), and capillene (6-phenyl-2,4-hexadiyne) (4.8%), which are two unusual and rarely occurring alkynes (*21*, *28*), whereas these alkynes are not found in Turkish tarragon oil (**Table 1**). The chemical composition of Iranian tarragon oil (*22*) contained (*E*)-anethole (21.2%), (*E*)-α-ocimene (20.6%), limonene (12.4%), α-pinene (5.1%), and *allo*-ocimene (4.8%) as predominant constituents. Interestingly, the chemical composition of Turkish tarragon studied in the present study was partly similar to that of Iranian tarragon oil (*22*). Previous researchers also showed that bornane derivatives and 1,8-cineole are major components of many essential oils of *Artemisia* species. Camphor, which is a bornane derivative, and 1,8-cineole were the main components of the essential oils of some *Artemisia* species (*11*, *18*, *23*). In contrast, these compounds were not detected in the oil of *A. dracunculus* oil in the present study.

Antioxidant and DPPH Radical Scavenging Activities of Tarragon Oil. The antioxidant activity of tarragon oil at 100 and 200 μg/mL concentrations was determined using the thiocyanate method. Antioxidant activity increased with increasing amount of the oil (**Figure 1a**). However, it showed a weak antioxidant activity in comparison to the activity of a 100 μg/mL concentration of BHT. Similarly, the DPPH radical scavenging activity of essential oil was lower than that of BHT (100 μg/mL) (**Figure 1b**). The weak antioxidant and DPPH radical scavenging activities of tarragon oil can be attributed to the absence of some components that have antioxidant activity. The oils isolated from plant species consist of the various constituents. Therefore, determination of the component(s) responsible for activity is very difficult.

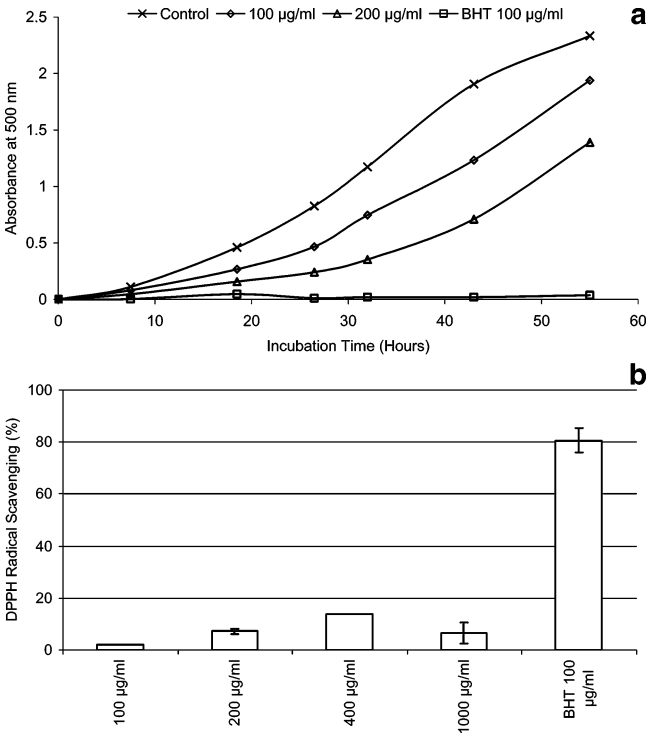

Figure 1. (**a**) Antioxidant activity of *A. dracunculus* essential oil (100 and 200 μg/mL) and BHT (100 μg/mL). (**b**) DPPH radical scavenging activity of *A. dranculus* essential oil (100, 200, 400, and 1000 μg/mL) and BHT (100 μg/mL).

Antifungal Activities of Essential Oils. The antifungal activities of the essential oils (20 μL/Petri dish) isolated from *A. absinthium*, *A. dracunculus*, *A. santonicum*, and *A. spicigera* were tested against phytopathogenic fungal species and compared with the commercial antifungal reagent benomyl (**Table 2**). Previously, we have reported the antifungal activities of 10, 20, and 40 μL doses of essential oils of Turkish *A. absinthium*, *A. santonicum*, and *A. spicigera* against 11 phytopathogenic fungi (*11*). Therefore, in the present study, these oils have not been tested against these 11 fungal species. However, the results given in our previous report (*11*) were also included in this study to compare the activities (**Table 2**). The growth of fungal species was significantly reduced by all of the essential oils tested ($p < 0.05$), and in many cases the growth of some fungal species was completely inhibited (**Table 2**). It was also interesting to find that the inhibition effects of the oils against some fungal species were higher than that of commercial benomyl. All of the oils completely inhibited the growth of *Sclerotinia* sp., whereas benomyl was not fungitoxic against this fungus. Nevertheless, *A. dracunculus* oil had weaker antifungal activity as compared with other *Artemisia* oils tested (**Table 2**). Weak antifungal activity of *A. dracunculus* may be due to its different major components and chemical composition in comparison to those of other *Artemisia* oils tested. Camphor, 1,8-cineole,

borneol, α-terpineol, terpinen-4-ol, and bornyl acetate were the major components of the oils of Turkish *A. santonicum* and *A. spicigera* (*11*). These oils contained relatively high proportions of oxygenated monoterpenes and showed also similar antifungal activities (*11*). Unlike *A. santonicum* and *A. spicigera* oils, *A. dracunculus* oil, which had a weaker antifungal activity, was composed of relatively lower proportions of oxygenated monoterpenes. Furthermore, the essential oils consist of complex mixtures of numerous constituents. Other major or minor compound(s) might cause the antifungal activity exhibited. Possible synergistic and antagonistic effects of compound(s) in the essential oil should also be taken into consideration.

Previous papers on the analyses and antifungal activities of essential oils of some species of various genera have shown that they have various degrees of growth inhibition effects against some phytopathogenic fungal species (*10*−*12*, *34*−*37*). On the basis of the results reported in these papers and unpublished data, it can be concluded that the essential oils rich in oxygenated monoterpenes have relatively higher antifungal activity.

In conclusion, the 20 μL doses of the oils of *A. santonicum*, *A. spicigera*, and *A. absinthium* as well as *A. dracunculus* were found to be fungitoxic against 34 agricultural pathogenic fungal species. The essential oils, in many cases, completely inhibited the growth of some fungal species. Therefore, it can be suggested that *A. santonicum*, *A. spicigera*, and *A. absinthium* oils may be used as antifungal reagents to protect plants against fungal diseases. However, further studies need to be conducted to determine the cost, efficacy, and safety of these oils.

Antibacterial Activities of Essential Oils. The inhibitory effects of *A. absinthium*, *A. dracunculus*, *A. santonicum*, and *A. spicigera* essential oils at 600, 900, and 1200 μg/disk concentrations on the growth of 64 bacterial strains including plant, food, and clinic origins were tested. The oils showed various degrees of antibacterial activities depending on tested bacterial strains (**Table 3**). Interestingly, there was no correlation between oil concentrations and antibacterial activities. The oils of *A. santonicum* and *A. spicigera* showed the weak antibacterial activity at a broader spectrum. However, *A. absinthium* and *A. santonicum* were active against a limited number of bacterial strains. Previously, we have reported that the essential oils of *A. santonicum* and *A. spicigera* species have similar chemical compositions and major components such as camphor, 1,8-cineole, borneol, terpinen-4-ol, bornyl acetate, and spathulenol (*11*). Thus, their similar antibacterial activities can be attributed to their similar chemical compositions. Contrarily, the oils of both *A. absinthium* and *A. dracunculus* are different in terms of chemical composition and major constituents as compared with *A. santonicum* and *A. spicigera* oils. The oil of *A. absinthium* consisted of mainly chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%), and caryophyllene oxide (4.3%) (*11*), whereas tarragon oil consists of mainly (*Z*)-anethole (81.0%), (*Z*)-β-ocimene (6.5%), (*E*)-β-ocimene (3.1%), and limonene (3.1%) (**Table 1**). Thus, different inhibitory effects of *A. dracunculus* and *A. absinthium* may be attributed to the differences in the biological properties of the main compounds in the oil. The wide antibacterial spectra of *A. santonicum* and *A. spicigera* oils may also be attributed to their relatively high content of oxygenated monoterpenes (*11*). Recently, oxygenated monoterpenes such as camphor, 1,8-cineole, terpinen-4-ol, and borneol, which were detected in the oils of *A. spicigera* and *A. santonicum*, were reported to exhibit antibacterial activity (*26*, *38*−*40*). These reports are compatible with our results in the present study.

Various microorganisms such as *Escherichia coli*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Acinetobacter* sp., *Bacillus* sp., *Brucella* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Staphylococcus* sp. have been reported as the causal agents of foodborne diseases and/or food spoilage (*41*). In the present study, the essential oils were also tested for antibacterial activities against some foodborne pathogens (**Table 3**). As shown in this table, the oils of *A. santonicum* and *A. spicigera* exhibited considerable antibacterial activities against the majority of foodborne pathogens. These oils were also active against bacterial strains that cause bacterial diseases in plants. It was interesting to find that essential oils were active against penicillin-resistant bacterial strains. For instance, the oils (especially *A. santonicum* and *A. spicigera* oils) showed inhibitory effect on the growth of *Xanthomonas axanopodas* pv. *vesicatoria*, *Bacillus megaterium*, *Enterobacter cloaceae*, *E. coli*, and *Klebsiella planticola*, which are penicillin-resistant bacteria.

Although there are numerous reports on the analyses of essential oils from *Artemisia* species in the literature, some *Artemisia* oils were tested against only a limited number of bacteria (*11*, *24*−*28*). However, a wide spectrum of antimicrobial activities (64 bacterial strains) of four *Artemisia* species are reported in the present study.

In conclusion, the development of natural antimicrobials will help to decrease the negative effects (residues, resistance, and environmental pollution) of synthetic drugs. In this respect, natural antimicrobials may be also effective, selective, biodegradable, and less toxic to environment. The oils of *A. santonicum* and *A. spicigera* had wide spectra of considerable antibacterial activities; besides, their inhibitory effects are low in comparison to penicillin. In view of the present results, it is concluded that these essential oils can be used as antimicrobial agents for therapy of plant diseases and infectious diseases and in food preservation. However, the safety and toxicity of these compounds will need to be addressed.

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