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Antimicrobial and antioxidant properties of *Artemisia* L. species from western Anatolia

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Abstract: *Artemisia* L. taxa (*A. absinthium* L., *A. arborescens* L., *A. campestris* L., *A. scoparia* Waldst. & Kit., *A. santonicum* L., and *A. vulgaris* L.) naturally distributed through western and southwestern Turkey were chosen as experimental materials in this study. Essential oils of the aerial parts of these *Artemisia* species were isolated by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The major components were identified as sabinene in *A. absinthium* (17.56%), camphor in *A. arborescens* (33.39%), 1,2-dehydro acenaphthylene in *A. campestris* and *A. scoparia* (20.71% and 11.80%, respectively), and α-thujone in *A. vulgaris* and *A. santonicum* (56.13% and 39.46%, respectively). Essential oils and methanolic extracts of the plants were tested for antimicrobial activity using the disk-diffusion method against 8 bacteria and 1 fungus. *Staphylococcus aureus* was the most sensitive bacteria to all of the essential oils. *A. santonicum* and *A. scoparia* were the most active plants against *Candida albicans*, with 35-mm and 40-mm diameter zones, respectively. Antioxidant capacities of the plants were also tested. The radical scavenging activity of *A. scoparia* extract (48.51%) and essential oil (80.08%), the Trolox equivalent antioxidant capacity of *A. campestris* extract (10.76 ± 0.47), and the α-tocopherol equivalent of *A. absinthium* extract (5.87 ± 0.17) were the highest results.

Key words: *Artemisia*, essential oil, antimicrobial activity, antioxidant activity

Batı Anadolu *Artemisia* L. türlerinin antimikrobiyal ve antioksidan özellikleri

Özet: Bu çalışmada Batı ve Güney-Batı Anadolu'da doğal yayılış gösteren *Artemisia* L. taksonları (*A. absinthium* L., *A. arborescens* L., *A. campestris* L., *A. scoparia* Waldst. & Kit., *A. santonicum* L., *A. vulgaris* L.) araştırma materyali olarak seçilmiştir. *Artemisia* türlerinin toprak üstü kısımlarından hidrodistilasyon yöntemi ile elde edilen uçucu yağlar, GC-MS ile analiz edilmiştir. Ana bileşikler *A. absinthium*'da sabinen (% 17,56), *A. arborescens*'de kafur (% 33,39), *A. campestris* ve *A. scoparia*'da 1,2 dihidroasenaftalen (sırasıyla % 20,71 ve % 11,80), *A. vulgaris* ve *A. santonicum*'da α-tuyon (sırasıyla % 56,13 ve % 39,46) olarak bulunmuştur. Uçucu yağlar ve metanollü ekstreler disk difüzyon yöntemi ile 8 bakteri ve 1 mantar suşuna karşı antibakteriyal etki açısından incelenmiştir. *Staphylococcus aureus* tüm uçucu yağlara en hassas bakteri olarak bulunmuştur. *A. santonicum* ve *A. scoparia*, *Candida albicans*'a karşı en etkili türler olarak tespit edilmiştir. Bitkilerin ayrıca antioksidan aktiviteleri de araştırılmıştır. *A. scoparia* uçucu yağı (% 80,08) ve ekstresinin (% 48,51) radikal süpürücü etkisi, *A. campestris* ekstresinin troloks eşdeğerliği antioksidan kapasitesi (10,76 ± 0,47) ve *A. absinthium* ekstresinin α-tokoferol eşdeğerliği antioksidan kapasitesi (5,8 ± 0,17) en yüksek bulunan sonuçlardır.

Anahtar sözcükler: *Artemisia*, uçucu yağ, antimikrobiyal aktivite, antioksidan aktivite

Introduction

The genus *Artemisia* L. is among the largest and most widely distributed genera of the family Asteraceae, consisting of 522 small herb and shrub species native to the northern hemisphere, South America, southern Africa, and the Pacific Islands (1,2).

These herbs have been used worldwide in folk medicine since ancient times (3,4). They have been used as tonics, antimalarials, antihelmintics, and antidiabetics, and in treating wounds, bronchitis, ulcers, and tuberculosis in traditional Anatolian medicine (5-8). There are also several reports concerning the antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial, and antifungal activities of different *Artemisia* species (1,9-12). "The chemical studies on *Artemisia* species indicate that all classes of compounds are present in the genus with particular reference to terpenoids and flavonoids. The rich accumulation of essential oils and other terpenoids in the genus is responsible for the use of various members for flavouring foods or liqueurs" (1).

Artemisia is represented by 23 species in the Turkish flora, and 6 of them are naturally distributed in western and southwestern Anatolia (13,14). These are *A. absinthium* L., *A. arborescens* L., *A. campestris* L., *A. scoparia* Waldst. & Kit., *A. santonicum* L., and *A. vulgaris* L. All of these taxa have been the subject of numerous chemical studies. Most of that research dealt with the essential oil composition of the plants and indicated that geographic origin, harvesting time, and environmental edaphic factors cause the variability of the composition of the oils (5,15-23). Even though the essential oil chemistry of *A. absinthium*, *A. santonicum*, *A. scoparia*, and *A. campestris*, originating from eastern and inner Anatolia, have been reported previously (24-26), there is no report of the essential oil composition of the western Anatolian *Artemisia* species.

This study was conducted to investigate the essential oil compositions and antimicrobial and antioxidant activities of the essential oils and methanol extracts of the western Anatolian *Artemisia* taxa mentioned above.

Material and methods

Plant material

The aerial parts of the *Artemisia* species were collected from different areas of western and southwestern Anatolia between the years 2003 and 2005 at the flowering stages. Voucher specimens were deposited in the herbarium of Ege University (IZEF), Faculty of Pharmacy, İzmir, Turkey (Table 1).

Preparation of the extracts

The aerial parts of the plants were dried under shade and powdered. Crude materials (50 g) were extracted successively with 1 L of methanol, using a Soxhlet extractor for 6 h. The solvent was evaporated to dryness under reduced pressure on a rotary evaporator. Dried extracts were stored at 4 °C until studied (Table 1).

Isolation of essential oils

The air-dried samples were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h to obtain the essential oils. They were dried over anhydrous sodium sulfate, filtered, and stored at 4 °C in darkness (Table 1).

Gas chromatography-mass spectrometry analysis

The analyses were carried out on a Shimadzu 2010 gas chromatography quadrupole mass spectrometry system fitted with an HP-5 column (60 m × 0.25 mm ID × 0.25 μm film thickness). The carrier gas was helium, with a linear velocity of 33.2 cm/s. Initial oven temperature was held at 50 °C for 3 min and then programmed to increase from 50 °C to 270 °C at 3 °C/min, and finally held isothermally for 10 min at 270 °C. The injection and ion source temperatures were 270 and 250 °C, respectively. The injection volume was 1 μL in the splitless mode. Masses were taken at 70 eV. The mass range was from 40 to 500 m/z.

Identification of the essential oil components

The components were identified by matching relative retention times and mass spectra with authentic samples from essential oil library data (Nist 27, Wiley 229, Szterp and Nist 147) and by comparing relative retention indices (RRI) with published data (27) (Table 2).

Table 1. Collection sites, dates, voucher specimens, and yields of *Artemisia* L. species.

	Turkish name	Locality	Date	Voucher specimen	Extract yield (%)	Oil yield (%)
A. absinthium	Pelinotu, Akpelin	Antalya, Alanya, 1514 m 37°47′10″N 28°56′05″E	29.07.2003	5657	17.78	1.1
A. arborescens	-	Muğla, Bodrum, 0 m	14.04.2005	5802	24.76	1.2
A. campestris	Kara yavşanotu	Denizli, Altındere, 850 m 37°47′10″N 28°56′05″E	30.09.2003	5665	21.96	0.7
A. scoparia	-	Manisa, Salihli, 112 m 38°28′46″N 28°03′56″E	30.09.2003	5662	17.16	0.9
A. santonicum	Deniz yavşani	Balıkesir, Edremit, 0 m 39°33′43″N 26°57′02″E	03.09.2003	5661	21.76	0.4
A. vulgaris	Ayvadana	Denizli, Başkarcı, 450 m 37°45′42″N 28°58′48″E	30.09.2003	5663	14.92	0.4

Antimicrobial activity

Microbial strains: In vitro antibacterial studies were carried out against 8 bacteria strains (*Staphylococcus aureus* ATCC 6538/P, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC 11230, and *Salmonella typhimurium* CMM 5445) and 1 fungus (*Candida albicans* ATCC 10239). All microorganism cultures were obtained from the American Type Culture Collection (ATCC) and the Microbiology Department's Culture Collection of Ege University, İzmir, Turkey.

The antimicrobial activity of the essential oils was tested using the disk-diffusion method (28-30). Briefly, filter paper disks, 6 mm in diameter, were impregnated with 20-30 µL of the essential oils (directly) and extracts (100 mg/mL). The bacteria strains were inoculated (5×10^6 cfu/mL) on tryptic soy agar (Oxoid) and the fungus strain was inoculated on Sabouraud dextrose agar (Oxoid), and all were incubated for 24 h. The agars were dispensed onto sterile plates, and then sterile disks were impregnated with the oils and extracts (4 disks for a 9-cm plate). The plates were incubated at 37 °C for 24 h (27 °C for *Enterobacter cloacae*). After incubation, all zones of growth inhibition and the diameters of the zones were measured in millimeters. All tests were done in triplicate and repeated 3 times. The results were expressed as average values. Ceftazidime (20 µg/disk) and ketaconazole (20 µg/disk) were used as positive controls (Table 3).

Antioxidant activity

Free radical scavenging activity assay (FRSA): DPPH-radical scavenging activity was tested according to the method described previously with some modifications (31). Test samples were prepared by dissolving 0.25 mg of dry extract in 4 mL of methanol and 1 µL of essential oil in 10 mL of hexane. A methanolic solution of DPPH (1 mM, 0.5 mL) was then added to the samples. The absorbances were determined spectrophotometrically at 517 nm. The measurements were carried out in triplicate. Butylated hydroxytoluene (BHT; 1 mg/mL) was used as a positive control. The inhibition percentages were calculated as follows:

Inhibition % = $[(A_B - A_A)/A_B] \times 100$, where A_B is the absorption of the blank and A_A is the absorption of the sample.

Table 2. The compositions of the essential oils of *Artemisia* L. species.

No.	Compound	RRI	AB (%)	AR (%)	AC (%)	AS1 (%)	AS2 (%)	AV (%)
1	α-Thujone	932	0.50	-	-	-	-	-
2	α-Pinene	941	0.84	1.37	2.79	0.12	1.37	-
3	β-Pinene		-	-	6.31	-	-	-
4	Camphene	957	-	2.50	-	0.17	2.63	-
5	Sabinene	980	17.56	-	-	1.41	-	-
6	Myrcene	994	10.96	1.49	-	0.15	-	-
7	α-Phellandrene	1008	5.37	-	-	-	-	-
8	α-Terpinene	1022	-	0.68	-	0.20	-	-
9	p-Cymene	1030	4.69	0.99	3.75	0.93	4.02	-
10	Limonene	1034	-	0.53	0.87	-	0.96	-
11	1,8-Cineole	1040	1.6	-	0.59	6.65	0.45	8.47
12	γ-Terpinene	1063	1.13	1.26	-	0.44	-	-
13	Linalool	1099	3.89	-	-	-	1.01	1.01
14	Isopentyl-2-methylbutanoate	1102	-	1.31	-	-	-	-
15	α-Thujone	1107	0.26	1.29	4.78	39.46	1.57	56.13
16	β-Thujone	1118	0.14	0.13	0.73	25.14	0.21	12.02
17	Allo-ocimene	1128	0.14	-	0.64	-	-	-
18	Myroxide	1132	5.76	-	-	-	-	-
19	cis-Sabinol	1143	1.59	-	-	-	-	-
20	Pinocarveol	1145	-	-	1.47	-	0.25	0.46
21	Camphor	1149	-	33.39	0.94	1.95	0.32	0.40
22	Sabina ketone	1165	-	-	-	0.55	-	-
23	Pinocarvone	1170	-	-	0.78	0.20	0.22	0.42
24	Borneol	1173	-	0.86	-	0.39	-	0.25
25	Terpin-4-ol	1183	1.44	4.75	0.52	0.91	-	0.24
26	α-Terpineol	1194	0.28	0.54	0.46	0.15	-	-
27	Myrtenol	1196	-	-	1.78	-	-	-
28	Methylchavicol	1198	-	-	-	-	1.38	-
29	Carvone	1249	-	-	-	0.13	-	0.46
30	Chrysantenyl acetate	1264	10.97	-	-	-	-	-
31	Perilla aldehyde	1280	-	0.43	-	-	-	-
32	1-phenyl-penta-2,4-diene	1289	-	-	2.06	-	1.34	-
33	trans-Sabinyl acetate	1292	7.71	-	-	-	-	-
34	Eugenol	1359	-	-	0.2	0.10	3.33	-
35	Methyl cinnamate	1383	-	-	-	-	1.21	-
36	β-Elemene	1390	-	-	-	-	0.79	-
37	Methyl eugenol	1398	-	-	5.49	-	4.65	-
38	Z-Jasmone	1398	-	-	-	2.18	-	-
39	Caryophyllene (E)	1417	-	2.05	-	0.29	1.34	0.94
40	1,2-dehydro acenaphthylene*	1488	-	-	20.71	-	11.80	-
41	β-Selinene	1483	-	054	-	1.98	-	-
42	Spathulenol	1576	0.44	0,34	6.47	0.79	15.55	1.39
43	Caryophyllene oxide	1581	0.34	4.35	1.08	0.17	11.44	10.19
44	Humulene epoxide	1608	-	0.37	-	-	1.06	1.27
45	α-Cadinol	1639	-	-	-	0.32	5.07	-
46	Capillin	1647	-	-	10.38	-	-	-
47	β-Eudesmol	1649	-	7.66	1.16	-	-	-
48	Isoeugenol acetate	1651	-	-	-	-	2.88	-
49	Chamazulene	1733	4.31	21.05	-	-	-	-
50	Tremetone	1740	-	-	15.83	-	-	-
	Total		79.78	87.32	89.79	84.78	74.85	94.50

RRI: Relative retention indices, AB: *A. absinthium* essential oil, AR: *A. arborescens* essential oil, AC: *A. campestris* essential oil, AS1: *A. santonicum* essential oil, AS2: *A. scoparia* essential oil, AV: *A. vulgaris* essential oil, *: comparison with authentic sample.

Table 3. Antioxidant activity results of *Artemisia* essential oils and extracts.

Plant material		RSA (%)	TEAC	TAC	TPC	TFC
A. absinthium	ME	28.43 ± 0	3.22 ± 0.13	5.87 ± 0.17	161.8 ± 1.41	57.39 ± 1.41
	EO	45.66 ± 0.87	-	2.89 ± 0.16	NT	NT
A. arborescens	ME	11.79 ± 0.46	1.67 ± 0.05	5.33 ± 0.11	100.8 ± 1.41	71.41 ± 5.40
	EO	NT	2.06 ± 0.22	3.39 ± 0.11	NT	NT
A. campestris	ME	38.00 ± 0.89	10.76 ± 0.47	2.39 ± 0.06	201.4 ± 1.41	93.86 ± 16.36
	EO	39.05 ± 0.96	-	-	NT	NT
A. santonicum	ME	47.62 ± 0.40	4.66 ± 0.02	2.78 ± 0.08	293.8 ± 0.02	109.02 ± 9.98
	EO	60.55 ± 0.52	-	-	NT	NT
A. scoparia	ME	48.51 ± 0.30	5.11 ± 0.09	2.85 ± 0.11	332.13 ± 15.8	121.96 ± 10.7
	EO	80.08 ± 1.57	-	-	NT	NT
A. vulgaris	ME	43.38 ± 1.09	4.40 ± 0.19	2.34 ± 0.21	217.46 ± 2.30	67.98 ± 6.65
	EO	70.24 ± 0.72	-	-	NT	NT

ME: Methanol extract, EO: essential oil, NT: not tested, -: not active. Results are mean ± SD of 3 replicate analyses.
RSA: Radical scavenging activity (FRSA of synthetic antioxidant BHT (1 mg/mL) was determined as 78.99 ± 0.25%) (ME: 0.25 mg/4 mL, EO: 1 µL/10 mL).
TEAC: Trolox equivalent antioxidant capacity (mM/mg for extracts and mM/L for essential oils) (TEAC of synthetic antioxidant of BHT was determined as 8.84 ± 0.31 mM/mg) (ME: 0.025 mg/mL, EO: 1 µL/10 mL).
TAC: Total antioxidant capacity (mM α-tocopherol equivalent) (ME: 0.1 mg/mL, EO: 1 µL/10 mL).
TPC: Total phenolic content (gallic acid equivalent mg/L) (ME: 0.1 mg/mL).
TFC: Total flavonoid content (quercetin equivalent µg/mL) (ME: 1 g/L).

Trolox equivalent antioxidant capacity (TEAC) assay: Antioxidant activity was measured using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method as described before (32). It is based on the Trolox equivalence of the samples. Mixed with 2.45 mM potassium persulfate was 7 mM ABTS, in order to produce an $ABTS^+$ radical. This radical was left in a dark room at 30 °C for 2 days to reach stable absorbance at 734 nm.

Extracts and essential oils were diluted in ethanol to a final concentration of 0.1 mg/mL. Ethanol was used as the blank. The $ABTS^+$ solution was diluted with 5 mM phosphate buffer until it had an absorbance of 0.70 ± 0.02 at 734 nm. Next, 1 mL of this stock solution was taken and mixed with 10 µL of a sample solution and measured at 734 nm for 1-6 min after the initial mixing. All measurements were carried out in triplicate. The inhibition percentages were calculated as follows:

Inhibition % = $(A_{ABTS}^{+} - A_{6.min}) \times 100/A_{ABTS}^{+}$, where $A_{ABTS.+}$ is the absorbance of $ABTS^+$ at 734 nm (0.700 ± 0.02) and $A_{6.min}$ is the 6-min absorbance after the addition of the sample to the $ABTS^+$.

A standard curve of Trolox was obtained using a Trolox standard solution at various concentrations (2.5-15 mM) in ethanol. The absorbances of the samples were compared to that of the standard curve and the antioxidant properties were expressed as mM Trolox equivalent/mg for extracts and mM/L for essential oils.

Total antioxidant capacity (TAC) assay: The phosphomolybdenum assay described by Prieto et

al. was used (33). Namely, 1 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was mixed with 10 µL of extract solutions (0.1 mg/mL) and essential oils (1 µL/10 mL) and left at 37 °C for 90 min. After cooling, the absorbance of the phosphomolybdenum complex was measured at 695 nm. Antioxidant capacities were expressed as α-tocopherol equivalents according to the standard curve of α-tocopherol obtained at various concentrations (1, 2.5, 5, 7.5, and 10 mM).

Determination of total phenolics: Determination of total phenolic content was performed according to the method of McDonald et al. (34) using Folin-Ciocalteu reagent. Briefly, 0.5 mL of each extract (0.1 mg/mL) was mixed with 5 mL of Folin-Ciocalteu reagent (1:10 with distilled water), and then 4 mL of 1 M Na_2CO_3 was added to the mixture and heated in a water bath at 45 °C for 15 min. The absorbances of the mixtures were measured at 765 nm. A standard curve was prepared using gallic acid in various concentrations (50, 100, 150, 200, and 250 mg/L). All measurements were carried out in triplicate and the results were expressed as gallic acid equivalents (mg/L) (Table 4).

Determination of total flavonoids: The total flavonoid contents of the methanolic extracts were determined by the aluminum chloride colorimetric method of Chang et al. (35) with some modifications. First, 0.5 mL of each extract (1 g/L) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. This was then incubated at room temperature for 30 min. The standard curve was prepared using quercetin in various concentrations (12.5, 25, 50, 75, and 100 µg/mL) with the same method. The absorbances of the mixtures were measured at 415 nm. All measurements were carried out in triplicate. Results were expressed as quercetin equivalents (µg/mL) (Table 4).

Table 4. Antimicrobial activity results of *Artemisia* essential oils and methanolic extracts (diameter zones of inhibition, mm).

Plant material		Microorganisms								
		SA	***SE***	***ST***	***PA***	***EF***	***ECL***	***ECO1***	***ECO2***	***CA***
A. absinthium	ME	-	-	-	9	-	-	10	7	-
	EO	18	9	8	-	8	8	8	9	23
A. arborescens	ME	-	-	-	21	-	-	7	8	-
	EO	7	8	-	14	7	7	7	8	18
A. campestris	ME	-	-	-	16	-	-	-	7	-
	EO	14	7	-	-	8	7	7	7	20
A. scoparia	ME	-	-	-	15	-	-	7	-	-
	EO	20	9	8	-	9	8	7	8	40
A. santonicum	ME	-	-	-	20	-	-	9	10	-
	EO	24	21	8	14	19	12	14	10	35
A. vulgaris	ME	-	-	-	23	-	-	7	7	-
	EO	23	13	10	-	14	8	11	10	20
Ceftazidime		12	14	12	22	11	13	15	18	NT
Ketoconazole		NT	NT	NT	NT	NT	NT	NT	NT	34

SA: *Staphylococcus aureus* (ATCC 6538/P), SE: *Staphylococcus epidermidis* (ATCC 12228), ST: *Salmonella typhimurium* (CCM5445), PA: *Pseudomonas aeruginosa* (ATCC 27853), EF: *Enterococcus faecalis* (ATCC29212), ECL: *Enterobacter cloacae* (ATCC13047), ECO1: *Escherichia coli* (ATCC29998), ECO2: *Escherichia coli* (ATCC11230), CA: *Candida albicans* (ATTC10239).
-: No inhibition zone, NT: not tested, ME: methanolic extract, EO: essential oil.

Results and discussion

The essential oils of the aerial parts of the plants were subjected to gas chromatography-mass spectrometry (GC-MS) analysis in order to determine their chemical constituents. As can be seen in Table 2, the major components of the plants were sabinene (17.56%), myrcene (10.96%), and chrysantenyl acetate (10.97%) in *A. absinthium*; camphor (33.39%) and chamazulene (21.05%) in *A. arborescens*; 1,2-dehydro acenaphthylene (20.71%), tremetone (15.83%), and capillin (10.38%) in *A. campestris*; α-thujone (39.46%) and β-thujone (25.14%) in *A. santonicum*; spathulenol (15.55%), 1,2-dehydro acenaphthylene (11.80%), and caryophyllene oxide (11.40%) in *A. scoparia*; and α-thujone (56.13%) and β-thujone (12.02%) in *A. vulgaris*.

Terpenoids are the most commonly studied class of metabolites of the genus *Artemisia*. The essential oil of *A. absinthium* is found in several pharmacopoeias and there have been numerous studies performed on it. Mainly 4 major components, β-thujone, cis-epoxyocimene, trans-sabinylacetate, and chrysantenyl acetate, have been described from *A. absinthium*, primarily depending on the origin of the plant (1,20). In our results, chrysantenyl acetate was the only compound that was consistent with oil originating in France (22). It is known that sabinene is the first bicyclic intermediate to arise in the biosynthetic pathways to the epimeric thujones, so the majority of this compound might be due to the stage of the collection. Kordali et al. (2005) described chamazulene as the main compound from the *A. absinthium* of eastern Anatolia (24). We found this compound in low amounts in *A. absinthium*, but as the main component of *A. arborescens*. It might be produced from the unstable sesquiterpene lactone artabsin during the hydrodistillation process (1,36). Both *A. campestris* and *A. scoparia* contain 1,2-dehydro acenaphthylene, which is a polyaromatic hydrocarbon (PAH), a ubiquitous class of environmental contaminants (37). It was first wondered whether there had been environmental pollution of the plants or wax contamination during the processing. However, identification of the same compound in 2 different species and a literature survey of this compound confirmed that this compound is indeed synthesized by the plant. It was also found in Italian *A. variabilis* essential oil and in the essential oil of residues of *A. scoparia* from India (38,39). Monoterpene thujone is one of the most characteristic compounds of *Artemisia* species, and we identified it in *A. santonicum* and *A. vulgaris* in high amounts. After comparing all of the species mentioned above with information from previous studies, we can confirm the idea that geographic origin has an important effect on the chemical compositions of *Artemisia* species.

The antioxidant assay results of extracts and essential oils of the *Artemisia* species are given in Table 3. DPPH was reduced with the addition of all extracts and essential oils. The essential oil of *A. arborescens* could not be tested because of its dense black color. The essential oils of *A. scoparia*, *A. vulgaris*, and *A. santonicum* showed FRSA results similar to those of the antioxidant BHT. Methanolic extracts of all plants had a Trolox equivalent antioxidant capacity when compared to BHT, but the essential oils showed no significant results. With the exception of *A. absinthium* and *A. arborescens* oils, they also did not have ammonium molybdate reducing power as an α-tocopherol equivalent.

It is well known that there is a relationship between antioxidant activity and the phenolic content of the plant extracts (40,41). Antioxidant properties of the methanolic extracts can be attributed to the phenolic and flavonoid contents of the *Artemisia* species (1). However, further studies should be performed for isolation and identification of the compounds of the extracts.

The results of antimicrobial activity tests done against 8 bacteria and 1 fungus strain using the disk-diffusion method are shown in Table 4. The essential oils of most of the species exhibited antimicrobial activity in variously sized zones of inhibition. The essential oil of *A. santonicum* inhibited the growth of all microorganisms tested, while *A. santonicum* and *A. vulgaris* produced inhibition zones greater than or equal to that of the standard antibiotic ceftazidime against *Staphylococcus aureus*, *Streptococcus epidermidis*, and *Enterococcus faecalis*. The medicinally important pathogen *Staphylococcus aureus* was the most sensitive bacteria to all of the essential oils. The essential oils of *A. santonicum* and *A. scoparia* showed an inhibition zone against

Candida albicans larger than that of the standard antifungal ketaconazole. Methanolic extracts of the plants showed an effect only on *P. aeruginosa* and *E. coli* strains. The most active extracts were *A. vulgaris*, *A. arborescens*, and *A. santonicum* for *P. aeruginosa* with diameter zones (23, 21, and 20 mm, respectively) very close to that of the standard antibiotic ceftazidime. Extracts showed moderate effects on *E. coli* strains. *A. absinthium* (10 mm) was the most active on *E. coli* (strain ATCC29998).

Akrout et al. (2010) reported the antimicrobial and antiradical activities of the essential oil of *A. campestris* originating from Tunisia (42). Their low observed antiradical effect is similar to that of our sample, but their observed antimicrobial activities were quite different. Tunisian essential oil showed a strong effect on *E. coli*, while *S. aureus* was the most sensitive bacteria in our samples. This difference might be due to the different essential oil compositions. Methanolic extracts of *A. campestris* were also evaluated for antibacterial properties by Naili et al. in 2010 (43). The extract was reported to have a strong effect on *S. aureus* and *Bacillus subtilis* strains. In our study, the *A. campestris* extract showed no significant effect on *S. aureus*. In a previous study, the ethanolic extracts and essential oils of *A. scoparia*, *A. absinthium*, *A. vulgaris* (from Erzurum), and *A. santonicum* (from Tekirdağ) were subjected to antimicrobial screening against 4 bacteria and *C. albicans*. Only *A. scoparia* oil was reported to have an effect against *E. coli* and *C. albicans* (44), whereas, in our study, all of the essential oils had stronger activity and showed broader spectrum results against all tested organisms and *C. albicans*. The essential oil of *A. scoparia* was also reported to have an antimicrobial effect on 15 oral bacteria when tested using the minimum inhibitory concentration method by Cha et al. (45). Limited reported effects on *S. epidermidis* and *E. coli* are similar to our results, but we report a higher effect on *S. aureus*. Ramezani et al. (2004) also reported that Iranian *A. scoparia* extract showed an inhibition zone (13.6 mm) against *S. aureus* but not against *P. aeruginosa* (46). This is the opposite of our results, in which *A. scoparia* extract affected *P. aeruginosa* (15-mm inhibition zone) but not *S. aureus*. *A. absinthium* was also investigated by Dülger et al. and showed activity, especially against *Bacillus* and *Salmonella* strains (47). A comparison of our results with those of previous studies shows that the locality of the plant material and the extraction procedure cause differences in the antimicrobial activity of the plants. The strong effects of the essential oils of *A. vulgaris* and *A. santonicum* are probably due to the high α-thujone content.

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