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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · APRIL 2005

Impact Factor: 2.91 · DOI: 10.1021/jf048429n · Source: PubMed

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Screening of Chemical Composition and Antifungal and Antioxidant Activities of the Essential Oils from Three Turkish *Artemisia* Species

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The compositions of essential oils isolated from the aerial parts of *Artemisia absinthium*, *Artemisia santonicum*, and *Artemisia spicigera* by hydrodistillation were analyzed by GC-MS, and a total of 204 components were identified. The major components of these essential oils were camphor (34.9−1.4%), 1,8-cineole (9.5−1.5%), chamazulene (17.8−nd%), nuciferol propionate (5.1−nd%), nuciferol butanoate (8.2−nd%), caryophyllene oxide (4.3−1.7%), borneol (5.1−0.6%), α-terpineol (4.1−1.6%), spathulenol (3.7−1.3%), cubenol (4.2−0.1%), β-eudesmol (7.2−0.6%), and terpinen-4-ol (3.5−1.2%). The antifungal activities of these essential oils were tested against 11 plant fungi and were compared with that of a commercial antifungal reagent, benomyl. The results showed that all of the oils have potent inhibitory effects at very broad spectrum against all of the tested fungi. Pure camphor and 1,8-cineole, which are the major components of the oils, were also tested for antifungal activity against the same fungal species. Unlike essential oils, these pure compounds were able to show antifungal activity against only some of the fungal species. In addition, the antioxidant and DPPH radical scavenging activities of the essential oils, camphor, and 1,8-cineole were determined in vitro. All of the studied essential oils showed antioxidant activity, but camphor and 1,8-cineole did not.

KEYWORDS: Compositae; *Artemisia*; essential oil; antifungal activity; antioxidant activity; chamazulene; camphor; 1,8-cineole; DPPH radical scavenging activity

INTRODUCTION

The genus *Artemisia*, small herbs and shrubs, is one of the largest and most widely distributed genera of the Compositae family (*1*, *2*). Members of this genus have a characteristic scent or taste, have botanical and pharmaceutical interest, and are used in the liqueur-making industry (*1*, *3*). There are about 22 species of *Artemisia* genus in Turkish flora (*1*, *2*). *A. absinthium* 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 (*1*). *A. absinthium* is also known locally as "pelin otu", "acı pelin", "ak pelin" and "büyük pelin" (*1*). *A. spicigera*, named locally as "yavşan", is widespread in middle and eastern Anatolia, at an altitude between 1000 and 2500 m (*1*, *2*). *A. santonicum*, which is known as "deniz yavşanı" and "kokulu yavşan", grows on sandy places and salted land in Turkey. *A. santonicum* has been used as antihelmintic and in the treatment of diabetes (*1*). *A. absinthium* and *A. vulgaris* are also used traditionally in the Philippines for skin diseases and ulcerative sores. The dried leaves of these species are also used to help induce more rapid healing of wounds and are used in the treatment of eczema and herpes (*4*).

Recently, there has been a growing interest in research concerning the possible use of plant extracts for pest and disease control in agriculture, that are less damaging to the human health and environment (*5*, *6*). In the control of plant disease, antimicrobial chemicals such as benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors are often used. However, there is a serious problem in the effective use of these chemicals due to the development of resistance by fungi (*7*). To overcome this problem, higher concentrations of these chemicals are used, but this increases the risk of high-level toxic residues in the products. Therefore, our interest focused on the analyses and effectiveness of essential oils (*8*, *9*).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in the cells by different means (*10*, *11*). Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (*12*). ROS and RNS may cause DNA damage that could lead to mutation (*13*). All aerobic organisms, including

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10.1021/jf048429n CCC: $30.25
Published on Web 02/10/2005

human beings, have antioxidant defenses that protect against oxidative damage (*14*). However, these natural antioxidant mechanisms can be inefficient and hence dietary intake of antioxidant compounds becomes important (*15*). Free radicals can also cause lipid peroxidation in foods that leads to their deterioration. Although there are some synthetic antioxidant compounds, there are also some concerns about the side effects of these compounds (*16*). Therefore, research into the determination of the natural sources of antioxidants and antioxidant potential of plants is important.

Numerous studies in the literature have reported on the analyses of the essential oil compositions from various species of *Artemisia* (*3*, *17*−*23*). Previously, our research group has also reported the essential oil composition of *A. spicigera*, growing in Erzurum region of Turkey, but not its antifungal and antioxidant activities (*17*). On the other hand, there is so far no report about the chemical composition of the essential oils and their antifungal and antioxidant activities of Turkish *A. santonicum* and *A. absinthium*. Thus, the aim of the present study was (a) to investigate the chemical composition of the essential oils isolated from the aerial parts of *A. absinthium*, *A. santonicum*, and *A. spicigera* from Turkey, (b) to assess the antifungal activity of the essential oils and of two major compounds (1,8-cineole and camphor) against a group of phytopathogenic fungi species, and (c) to determine the antioxidant and DPPH radical scavenging activities of the essential oils and of 1,8-cineole and camphor, in vitro.

EXPERIMENTAL PROCEDURES

Plant Materials. The aerial parts of *A. santonicum* (ATA-9772), *A. spicigera* (ATA-9773), and *A. absinthium* (ATA-9774) were collected in the Erzurum region of Turkey in July 2003 at the flowering stages and were dried in shade. The voucher specimens have been deposited in the herbarium of Ataturk 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 in a sealed vial until use at 20 °C. Hydrodistillation of *A. absinthium*, *A. santonicum*, and *A. spicigera* yielded 0.67, 0.85, and 0.60% (w/w) of essential oils, respectively. The yields were based on dry materials of plant samples.

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

The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on SGE-BPX5 capillary column, and by matching of their mass spectra of peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data (*25*, *26*). Authentic samples were purchased from Sigma, Fluka, Alfa, or Aldrich.

Fungal Species and Antifungal Activity Assays. The agricultural pathogenic fungi were obtained from the culture collection at Ataturk 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 were *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium sambucinum*, *Fusarium solani*, *Penicillium jensenii*, *Penicillium* spp., *Rhizoctania solani*, *Sclerotium minor*, *Sclerotium sclerotiorum*, *Verticillium albo-atrum*, and *Verticillium tenerum*.

Antifungal activity was studied by using contact assay (in vitro), which produces hyphal growth inhibition (*8*). Briefly, PDA plates were prepared using 9 cm diameter glass Petri dishes; 10, 20, and 40 μL of the essential oils, 20 μL of 1,8-cineole (Sigma), and 12 mg of camphor (Fluka) were added to each of the PDA plates containing 20 mL of agar. Disks (5 mm diameter) of the fungal species were cut from 1-week-old cultures on PDA plates, and then mycelial surface of the disk was placed upside down on the center of dish. Therefore, fungal was contacting to growth medium on dish. Then, the plates were incubated in the dark at 22 ± 2 °C. After 6 days of inoculation of fungi species, the extension diameter (mm) of hyphae from centers to the sides of dishes was measured at 24 h intervals. Mean growth measurements were calculated from three replicates of each of the fungal species. PDA plates treated with distilled water, without essential oil or pure major compound solutions, were used as negative control. In addition, PDA plates treated with benomyl (12.0 mg/Petri dishes) were used as positive controls (*27*).

Growth inhibition of treatment against control was calculated by percentage, using the equation

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

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

Determination of Antioxidant Activity. The antioxidant activity was determined according to the thiocyanate method (*28*). Briefly, stock solutions of test samples were prepared at 25 mg/mL concentration in ethanol. Required stock solutions were mixed with 2.5 mL of 0.02 M linoleic acid (Fluka) emulsion [contains 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 oils and pure compounds were 100 μg/mL. BHT (butylated hydroxytoluene, Sigma) was used as positive control (25 or 50 μg/mL). The amount of peroxide was determined by measuring 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. The 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 (*28*). 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 and BHT (positive control) were 100, 200, and 400 μg/mL. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as % 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 the SPSS 10.0 software package. Values of $p < 0.05$ were considered to be significantly different.

RESULTS AND DISCUSSION

Essential Oil Compositions. Essential oil compositions and their relative amounts (RA) are shown in **Table 1**. As can be seen, *A. santonicum* and *A. spicigera* oils were similar in terms of most major volatile components, for example, camphor and cineole; borneol, terpinen-4-ol, α-terpineol, spathulenol, and caryophyllene oxide are predominant volatile components. β-Eudesmol (7.2% RA) and cubenol (4.2% RA) were the major constituents of the volatile constituents of *A. santonicum* oil, whereas the amounts of these compounds in the oil of *A. spicigera* relative to other examined volatiles were lower (0.6 and 0.2% RA, respectively). Both of these oils also contained relatively high amounts of oxygenated monoterpenes (68.6 and 51.5% RA, respectively). In contrast to the other two oils, the examined volatiles of *A. absinthium* oil exhibited a different chemical composition. It contained high amounts of chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%), and caryophyllene oxide (4.3%). The volatiles of *A. absinthium* oil were also characterized quantitatively by high amount of aromatic compounds (40.9% RA) and a low proportion of oxygenated monoterpenes (23.5% RA) as compared with the other oils. In addition, the proportions of camphor (1.4% RA) and 1,8-cineole (1.5% RA) in the oil of *A. absinthium* were lower than those in *A. santonicum* and *A. spicigera* oils. However, all oils were qualitatively rich in sesquiterpenes: in *A. santonicum*, 30.9%; in *A. absinthium*, 25.9%; in *A. spicigera*, 14.7% of total essential oil (**Table 1**).

Previous research showed that bornane derivatives and 1,8-cineole are major characteristic components of many species of *Artemisia* genus. Camphor (a bornane derivative) and 1,8-cineole were the major constituents of the essential oils of *A. asiatica* (*3*), *A. austriaca* (*17*), *A. afra* (*20*), *A. diffusa* (*22*), and *A. annua* (*29*). In the present study, the percentage amounts of some components in *A. spicigera* oil differ from those of our previous study (*17*). This may be due to analysis conditions. It has been previously reported that the oils of *A. absinthium* of French origin contain (*Z*)-epoxyocimene and chrysanthenyl acetate as major components, whereas the oils of Croatian *A. absinthium* contain mainly (*Z*)-epoxyocimene and β-thujone (*21*). Unlike in these papers, epoxyocimene is not detected and (*Z*)- and (*E*)-chrysanthenyl acetates and (*Z*)- and (*E*)-thujones are found in traces amounts in the Turkish *A. absinthium* in the present study (**Table 1**).

Artemisia species generally contain 1,8-cineole and bornane derivatives, which are widely used in the liqueur-making industry in many countries of the world. However, thujone derivatives, which are toxic components (*24*), are frequently found in the oils of many *Artemisia* species (*19*−*21*, *23*, *24*, *29*). The essential oil analyses of *A. santonicum* and *A. spicigera* showed that these oils contain mainly camphor, 1,8-cineole, and no thujone derivatives. Thus, the oils of *A. santonicum* and *A. spicigera* may be used in the liqueur-making industry. Unlike *A. santonicum* and *A. spicigera* oils, *A. absinthium* oil contains thujone derivatives in small proportions including (*Z*)-thujone and (*E*)-thujone at 0.2 and 0.5% RA, respectively.

Antifungal Activity of Essential Oils. The results of antifungal activity assays showed that the oils of *Artemisia* species had the inhibitory effects on the growth of fungi at a broad spectrum. The growth of all fungi species was reduced significantly by 20 and 40 μL doses of all of the tested oils, and in many cases the growth of some fungal species was completely inhibited (**Table 2**). However, the oil of *A. santonicum* was effective at 40 μL dose against *F. solani* but was not effective at the 20 μL dose. It was also interesting to find that, in many cases, the inhibition effects of *A. santonicum* and *A. spicigera* oils on the growth of fungi tested were higher than that of commercial benomyl.

In general, there was a correlation between the antifungal activity and the percentage of some major components. Camphor, 1,8-cineole, borneol, α-terpineol, terpinen-4-ol, bornyl acetate, and chrysanthenol were the major constituents of the oils of *A. santonicum* and *A. spicigera*. Both of these oils also contained the high proportion of oxygenated monoterpenes (**Table 1)**. These oils showed similar antifungal properties (**Table 2**). Unlike *A. santonicum* and *A. spicigera* oils, the oil of *A. absinthium* had weaker antifungal activity and was composed of relatively lower proportion of oxygenated monoterpenes (**Table 1**).

Commercially obtained camphor and 1,8-cineole exhibited weaker activity against the fungal species tested than did *Artemisia* oils (**Table 2)**. Whereas camphor and 1,8-cineole showed antifungal activity against limited fungal species, essential oils inhibited the growth of many fungi species. Although these compounds did not show strong antifungal activity against the fungal species, the oils of *A. santonicum* and *A. spicigera*, containing mainly camphor and 1,8-cineole, showed potent antifungal activities. The volatile oils consist of complex mixtures of numerous components. Other major or trace compound(s) might give rise to the antifungal activity exhibited. Possible synergistic and antagonistic effect of compound(s) in the 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 varying degrees of growth inhibition effects against some agricultural pathogenic fungal species (*6*, *8*, *9*, *30*−*33*). Likewise, it has been shown that pure 1,8-cineole exhibited a moderate but variable degree of antifungal activity against agricultural pathogenic fungal species (*35*). These reports are compatible with the present results. In addition to these papers, we have recently reported the antifungal activities of pure commercial compounds against five *Fusarium* species, *F. oxysporum*, *F. culmorum*, *F. sambucinum*, *F. solani*, and *F. acuminatum* (*8*). Although the compounds exhibited varying degrees of antifungal activity, β-caryophyllene oxide was very fungitoxic against the studied *Fusarium* species (8). The present study indicates that *Artemisia* essential oils contain β-caryophyllene oxide (**Table 1**). Therefore, β-caryophyllene oxide, detected in *Artemisia* oils, may affect their exhibited antifungal activities

In conclusion, the oils of *A. spicigera* and *A. santonicum* showed antifungal activities against 11 fungal species at 20 and 40 μL/dish concentrations. The essential oils completely stopped the growth of some fungal species, and in many cases their inhibition effects were stronger than that of commercial benomyl. Therefore, it can be suggested that the essential oils of *A. spicigera* and *A santonicum* may be used as antifungal reagents to protect plants against fungal diseases. However, further studies need to be conducted to evaluate these reagents above-mentioned on the wide range of phytopathogenic fungi.

Antioxidant and DPPH Radical Scavenging Activities of Essential Oils. Antioxidant activity of essential oils of *A. absinthium*, *A. santonicum* and *A. spicigera* was evaluated using the thiocyanate method. The highest antioxidant activity was shown by *A. santonicum* oil (**Figure 1a**). However, there were slight differences between antioxidant activities of *A. santonicum* and *A. absinthium* oils, and the differences between them were not statistically significant ($p > 0.05$). Nevertheless, these oils contain different major components (**Table 1**). To evaluate the

Table 1. Composition[a] of the Volatile Oils Isolated from *A. absinthium*, *A. santonicum*, and *A. spicigera*

no.	RI[b]	constituent	*A. absintihum* (%)	*A. santonicum* (%)	*A. spicigera* (%)	identification methods[c]
1	907	α-thujene	–[d]	0.1	–	MS
2	918	α-pinene	–	0.6	–	GC, MS
3	942	camphene	–	1.0	0.1	GC, MS
4	970	5-methyl-3-hexen-2-one	–	–	0.3	MS
5	971	sabinene	–	0.1	–	GC, MS
6	977	β-pinene	–	0.2	–	GC, MS
7	985	3-octanone	–	0.1	–	GC, MS
8	991	β-myrcene	0.2	0.5	0.1	GC, MS
9	1013	α-phellandrene	–	–	0.3	GC, MS
10	1022	α-terpinene	–	0.3	0.2	GC, MS
11	1033	*p*-cymene	0.6	0.4	0.5	GC, MS
12	1036	limonene	0.1	0.2	0.7	GC, MS
13	1041	1,8-cineole	1.5	7.5	9.5	GC, MS
14	1064	(*E*)-β-ocimene*[e]	0.1	–	–	MS
15	1065	*Artemisia* ketone	–	–	0.1	MS
16	1066	γ-terpinene	–	0.5	–	GC, MS
17	1079	(*Z*)-sabinene hydrate	–	1.0	–	MS
18	1079	(*E*)-arbuscolene	–	–	0.3	MS
19	1080	(*Z*)-linalol oxide	0.4	–	–	MS
20	1083	*Artemisia* alcohol	–	–	0.1	MS
21	1083	camphenilone	–	–	0.4	MS
22	1087	fenchone	0.1	–	–	GC, MS
23	1089	α-pinene oxide	–	–	0.1	MS
24	1090	terpinolene	–	0.1	–	MS
25	1091	(*E*)-linalol oxide	0.3	–	–	MS
26	1095	*p*-cymenene	0.1	–	–	MS
27	1103	pentyl butyrate	–	0.1	–	MS
28	1105	(*E*)-sabinene hydrate	2.9	–	0.7	MS
29	1105	linalool	0.2	0.6	0.4	GC, MS
30	1108	(*Z*)-thujone	0.2	–	–	GC, MS
31	1111	isopentylisovalerate	–	0.3	–	MS
32	1120	(*E*)-vertocitral C	–	1.2	–	MS
33	1122	(*E*)-thujone	0.5	–	–	GC, MS
34	1127	(*Z*)-*p*-mentha-2-en-1-ol	0.2	0.3	1.7	MS
35	1130	α-campholenal	0.1	0.1	–	MS
36	1131	terpinen-1-ol	–	–	0.3	MS
37	1136	nopinone	–	–	0.1	MS
38	1137	isocyclocitral	–	0.5	–	MS
39	1139	(*E*)-pinocarveol	0.7	–	1.2	MS
40	1140	(*E*)-*p*-mentha-2-en-1-ol	–	–	1.4	MS
41	1141	(*E*)-sabinol	–	0.4	–	MS
42	1143	(*Z*)-verbenol	0.3	0.3	–	GC, MS
43	1149	camphor	1.4	18.2	34.9	GC, MS
44	1150	neo-3-thujanol	0.1	–	–	MS
45	1152	isoborneol	–	0.1	–	MS
46	1153	sabinaketone	1.2	–	0.3	MS
47	1156	(*Z*)-chrysanthenol	–	2.0	1.3	MS
48	1163	(*E*)-*p*-terpineol	0.1	–	–	GC, MS
49	1165	borneol	0.6	4.0	5.1	GC, MS
50	1169	terpinen-4-ol	1.8	3.5	1.2	GC, MS
51	1175	isomenthol	0.2	–	0.2	GC, MS
52	1175	*p*-cymen-8-ol	1.7	0.2	1.0	MS
53	1180	α-terpineol	2.4	4.1	1.6	GC,MS
54	1188	(*Z*)-piperitol	–	0.1	–	MS
55	1188	myrtenol	tr[f]	–	0.6	GC, MS
56	1189	(*E*)-4-decenal	–	–	0.1	MS
57	1189	verbanol	–	–	0.9	MS
58	1189	D-verbenone	0.1	0.1	0.3	GC,MS
59	1191	isodihydrocarveol	–	0.1	–	MS
60	1195	(*E*)-pulegol	–	0.3	–	GC, MS
61	1195	(*E*)-carveol	0.1	–	0.2	GC, MS
62	1195	(*Z*)-sabinene hydrate acetate	–	–	0.3	MS
63	1196	nerol	0.5	0.1	–	GC, MS
64	1197	isobornyl formate	0.1	–	0.3	MS
65	1197	fenchyl acetate	0.1	–	–	MS
66	1198	(*E*)-chrysanthenyl acetate	0.1	0.8	0.1	MS
67	1211	cuminaldehyde	0.9	–	0.5	GC, MS
68	1211	neral	–	0.3	–	MS
69	1213	piperitone	0.1	–	2.6	MS
70	1214	(*Z*)-chrysanthenyl acetate	0.1	1.3	–	MS
71	1221	nonanoic acid	0.1	–	0.1	MS
72	1223	geranial	0.1	0.1	–	GC, MS
73	1224	neoisopulegol acetate	–	–	0.2	MS

Table 1. (Continued)

no.	RI[b]	constituent	*A. absintihum* (%)	*A. santonicum* (%)	*A. spicigera* (%)	identification methods[c]
74	1228	isopulegol acetate	–	–	0.2	MS
75	1230	bornyl acetate	0.3	2.2	1.0	GC, MS
76	1234	*p*-cymen-7-ol	0.3	–	0.5	MS
77	1235	lavandulyl acetate	–	0.7	–	MS
78	1236	thymol	0.3	0.3	0.6	GC, MS
79	1238	phenyl 2-methylpropionate	–	0.1	–	MS
80	1240	carvacrol	0.5	0.1	0.3	GC, MS
81	1242	myrcenyl acetate	–	0.1	–	MS
82	1244	iso-3-thujyl acetate	–	–	0.1	MS
83	1246	6-hydroxycarvotanacetone	–	–	0.1	MS
84	1249	dihydrocarveol acetate	–	–	0.3	MS
85	1252	2,4,6-trimethyl acetophenone	0.2	–	–	MS
86	1253	hexyl isovalerate	–	–	0.1	MS
87	1254	isodihydrocarvyl acetate	–	–	0.1	MS
88	1255	(*E*)-carveol acetate	0.4	0.1	0.2	GC, MS
89	1261	α-terpineol acetate	–	0.1	0.3	MS
90	1263	α-longipinene	–	0.1	–	GC, MS
91	1266	eugenol	0.3	0.8	0.1	GC, MS
92	1269	α-yılangene	0.1	–	0.1	MS
93	1273	α-copaene	0.2	0.1	0.1	GC, MS
94	1275	isobornyl propionate	–	0.1	–	MS
95	1275	(*E*)-myrtanol acetate	–	–	0.1	MS
96	1275	geranyl acetate	–	0.2	–	GC, MS
97	1276	β-bourbonene	0.1	–	0.1	MS
98	1278	β-cubebene	0.5	–	tr	MS
99	1279	β-elemene	–	0.4	–	MS
100	1281	benzene pentanoic acid methyl ester	–	0.1	–	MS
101	1285	(*Z*)-jasmone	–	0.6	–	GC, MS
102	1286	(*Z*)-isoeugenol	0.1	0.4	0.6	MS
103	1286	β-isocomene	0.2	–	–	MS
104	1287	(*Z*)-caryophyllene	0.2	–	0.3	GC, MS
105	1289	phenyl hexanal*	–	0.1	–	MS
106	1291	α-cedrene	0.8	–	–	GC, MS
107	1292	(*Z*)-*threo*-davanofuran	–	–	0.1	MS
108	1293	β-caryophyllene	1.1	1.2	0.4	GC, MS
109	1296	*p*-cymen-7-ol acetate	–	–	0.1	MS
110	1297	β-gurjunene	0.1	–	–	GC, MS
111	1301	αromadendrene	0.1	–	–	GC, MS
112	1303	2,3-dihydro-2,2,4,6-tetramethylbenzofuran	0.2	–	–	MS
113	1305	(*Z*)-β-farnesene	–	0.1	0.1	MS
114	1307	α-humulene	0.1	0.1	tr	GC, MS
115	1311	α-patchulene	–	0.1	–	MS
116	1311	cyclamen aldehyde	0.1	–	–	MS
117	1313	γ-gurjunene	–	0.1	–	GC, MS
118	1314	β-chamigrene	0.1	0.4	0.2	MS
119	1315	γ-muurolene	0.2	0.1	–	GC, MS
120	1317	linaly isobutyrate	1.4	–	–	MS
121	1317	germacrene-D	–	1.3	–	MS
122	1318	cyclogeraniol acetate	–	–	0.1	MS
123	1318	(*E*)-β-ionene	–	–	0.1	MS
124	1318	β-selinene	2.0	–	–	MS
125	1320	neryl isobutyrate	0.8	–	–	MS
126	1321	α-selinene	–	2.4	0.5	MS
127	1322	bicyclogermacrene	–	0.5	–	MS
128	1320	neryl butyrate	0.5	–	–	MS
129	1323	benzyl tiglate	–	–	0.1	MS
130	1324	3-methyl-2-phenylbutanoic acid ethyl ester	–	–	0.3	MS
131	1326	(*Z*)-α-bisabolene	0.2	–	0.4	MS
132	1326	(*E*,*E*)-α-farnesene	–	0.1	0.2	MS
133	1328	γ-cadinene	–	–	0.1	MS
134	1329	geranyl isobutyrate	2.3	0.1	–	MS
135	1330	cubebol	0.1	–	0.9	MS
136	1332	(*Z*)-calamenene	–	0.1	0.2	MS
137	1333	artedauglasia oxide-C	–	–	0.6	MS
138	1335	dehydro-*ar*-γ-himachalene	0.1	–	–	MS
139	1340	(*Z*)-nerolidol	0.2	–	–	MS
140	1341	α-calacorene	0.3	0.1	0.3	MS
141	1343	(*Z*)-sesquisabinenehydrate	2.7	–	–	MS
142	1343	elemol	–	0.1	–	MS
143	1346	germacrene-B	–	0.1	–	MS
144	1347	geranyl *n*-butyrate	1.7	–	–	MS
145	1347	(*E*)-nerolidol	–	0.1	0.3	GC, MS
146	1356	spathulenol	1.8	1.3	3.7	MS

Table 1. (Continued)

no.	RI[b]	constituent	*A. absintihum* (%)	*A. santonicum* (%)	*A. spicigera* (%)	identification methods[c]
147	1359	caryophyllene oxide	4.3	1.7	1.8	GC, MS
148	1360	globulol	–	–	0.2	MS,
149	1361	β-phenylethyl tiglate	–	–	0.3	MS
150	1363	gleenol	0.2	0.1	0.2	MS
151	1364	davanone	0.1	0.1	–	MS
152	1365	viridiflorol	0.3	–	0.3	MS
153	1377	(*Z*)-sesquilavandulol	0.4	0.4	0.8	MS
154	1381	α-acorenol	0.3	–	–	MS
155	1382	γ-eudesmol	–	1.0	–	MS
156	1382	(*E*)-sesquilavandulol	–	–	0.3	MS
157	1383	β-acorenol	0.2	–	–	MS
158	1383	epi-α-cadinol	0.4	–	–	MS
159	1384	cubenol	0.1	4.2	0.2	MS
160	1386	cedr-8(15)-en-9-α-ol	0.4	0.5	0.5	MS
161	1388	α-muurolol	0.1	–	0.2	MS
162	1390	vulgarone-B	0.2	–	–	MS
163	1392	(*Z*)-methyl jasmonate	–	–	0.4	MS
164	1396	β-eudesmol	1.1	7.2	0.6	MS
165	1397	α-cadinol	0.2	–	–	MS
166	1398	longipinocarvone	–	–	0.3	MS
167	1400	7-epi-α-eudesmol	1.3	–	0.1	MS
168	1403	14-hydroxy-9-epi-(*E*)-caryophhylene	0.7	0.2	0.5	MS
169	1408	α-bisabolene oxide	–	0.1	–	MS
170	1409	α-bisabolol	0.4	1.0	0.3	MS
172	1410	epi-α-bisabolol	–	0.1	0.2	MS
173	1417	(*Z*,*E*)-α-bergamatol	–	0.1	0.1	MS
174	1420	geranyl tiglate	–	0.3	–	MS
175	1421	*n*-heptadecane	–	–	0.1	MS
176	1421	14-hydroxy-α-humulene	–	0.2	–	MS
177	1423	chamazulene	17.8	0.3	–	MS
178	1425	(*E*,*Z*)-farnesol	–	0.4	–	MS
179	1426	α-bisabolol oxide A	–	0.3	–	MS
180	1431	benzyl benzoate	–	0.7	–	MS
181	1432	lanceol*	0.5	0.1	–	MS
182	1432	guaiazulene	0.9	–	–	GC, MS
183	1433	(*E*)-α-atlantone	0.2	–	–	MS
184	1434	14-hydroxy-α-muurolene	–	–	0.1	MS
185	1434	(*Z*,*E*)-farnesyl acetate	–	2.5	–	GC, MS
186	1438	(*E*,*E*)-farnesyl acetate	–	0.1	–	GC, MS
187	1439	hexahydrofarnesyl acetone	1.2	0.1	0.1	MS
188	1442	diisobuthyl phthalate	1.3	0.3	0.2	MS
189	1444	(*Z*)-nuciferol acetate	0.3	–	–	MS
190	1445	(*Z*)-lanceol acetate	0.5	–	–	MS
191	1447	farnesyl propionate*	0.6	0.1	–	MS
192	1450	nuciferol propionate*	5.1	2.1	–	MS
193	1450	lanceol propionate*	0.9	1.3	–	MS
194	1451	dipentyl phthalate	0.9	–	–	MS
195	1452	*n*-hexadecanoic acid	–	–	0.3	MS
196	1453	cedrane-8,13-diol	0.2	–	–	MS
197	1454	*n*-nonadecane	0.6	–	–	MS
198	1454	nuciferol butanoate*	8.2	0.5	–	MS
199	1455	lanceol butanoate*	0.2	0.3	–	MS
200	1456	cembrene	0.3	–	–	MS
201	1460	lanceol pentanoate*	1.2	–	–	MS
202	1462	phytol*	0.6	0.1	0.2	GC, MS
203	1463	nuciferol hexanoate*	0.7	–	–	MS
204	1465	ethyl hexadecanoate	0.4	–	–	MS
grouped components						
		monoterpene hydrocarbons (%)	0.4	3.6	1.4	
		oxygenated monoterpenes (%)	23.5	50.9	68.6	
		sesquiterpene hydrocarbons (%)	6.0	7.1	2.5	
		oxygenated sesquiterpenes (%)	19.9	23.9	12.2	
		diterpenes (%)	0.9	0.1	0.2	
		aromatics (%)	40.9	6.3	4.7	
		others (%)	2.3	1.2	1.9	
		total identified (%)	93.9	93.0	91.5	

[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 based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data (*25*, *26*). [d] Not detected. [e] An asterisk (*) indicates that the exact isomer was not identified. [f] Less than 0.1%.

effect of major compounds in antioxidant and DPPH radical scavenging activities, the antioxidant activities of commercially obtained camphor and 1,8-cineole were also studied. Neither of these compounds shows antioxidant activity at 100 μg/mL

Table 2. Antifungal Activities of Essential Oils of *Artemisia* Species, Camphor, and 1,8-Cineole

	mycelial growth[a] (mm)											
	A. absinthium			*A. santonicum*			*A. spicigera*		camphor	1,8-cineole	benomyl	
fungal species	10 μL	20 μL	40 μL	10 μL	20 μL	40 μL	10 μL	20 μL	40 μL	12 mg	20 μL	12 mg	control
A. alternata	28.9 ± 3.2	24.8 ± 3.0	14.1 ± 1.7***	19.8 ± 2.5**	14.1 ± 1.8***	5.0 ± 0.0***	25.4 ± 3.2	11.3 ± 1.5***	5.0 ± 0.0***	27.5 ± 3.5	27.1 ± 3.0	18.2 ± 1.6***	28.6 ± 3.2
F. oxysporum	28.0 ± 4.1	18.0 ± 2.1***	16.7 ± 2.3***	22.7±3.0**	12.8 ± 1.4***	7.5 ± 0.7***	28.0 ± 3.7	12.7 ± 1.5***	7.4 ± 0.8***	27.7 ± 4.0	27.9 ± 3.6	5.2 ± 0.1***	34.4 ± 4.6
F. sambucinum	31.2 ± 4.4***	28.4 ± 3.8***	22.0 ± 3.0***	18.2 ± 2.6***	9.3 ± 1.0***	8.1 ± 0.9***	20.4 ± 3.4***	10.1 ± 1.2***	5.7 ± 0.4***	25.9 ± 3.7***	29.0 ± 4.0***	5.0 ± ±0.0***	46.2 ± 6.1
F. solani	26.7 ± 3.1	20.1 ± 2.2	21.2 ± 2.8	23.7 ± 2.8	20.9 ± 2.5	13.4 ± 1.7***	26.9 ± 3.2	17.2 ± 2.2*	12.2 ± 1.5***	26.7 ± 3.3	18.8 ± 2.2	5.2 ± 0.1***	24.7 ± 2.9
P. jensenii	9.4 ± 1.0***	5.8 ± 0.3***	5.0 ± 0.0**	5.0 ± 0.0***	5.0 ± 0.0***	5.0 ± 0.0***	8.8 ± 0.8***	6.0 ± 0.4***	5.0 ± 0.0***	10.3 ± 1.1*	8.9 ± 0.8*	5.0 ± 0.0***	12.4 ± ±1.3
Penicillium spp.	15.9 ± 1.8	5.1 ± 0.1***	12.7 ± 1.3**	5.1 ± 0.1***	5.1 ± 0.1***	7.6 ± 0.6***	11.6 ± 1.1***	9.9 ± 0.9***	5.4 ± 0.2***	13.3 ± 1.5**	16.7 ± 2.1	6.3 ± 0.3***	17.3 ± 2.0
R. solani	34.6 ± 5.5***	14.6 ± 2.8***	13.8 ± 2.5***	10.3 ± 1.6***	5.0 ± 0.0***	5.0 ± 0.0***	18.3 ± 3.3***	5.1 ± 0.1***	5.0 ± 0.0***	26.3 ± 4.9***	52.8 ± 8.1	5.0 ± 0.0***	54.9 ± 7.5
S. minor	16.3 ± 2.7***	5.0 ± 0.0***	6.6 ± 1.1***	7.4 ± 0.9***	5.0 ± 0.0***	5.0 ± 0.0***	7.4 ± 1.0***	5.0 ± 0.0***	5.0 ± 0.0***	14.9 ± 2.2***	39.8 ± 8.1	5.0 ± 0.0***	38.4 ± 8.2
S. sclerotiorum	58.1 ± 8.6	5.0 ± 0.0***	16.8 ± 4.0***	43.1 ± 9.1*	5.0 ± 0.0***	5.0 ± 0.0***	44.2 ± 9.2*	10.1 ± 1.2***	5.0 ± 0.0***	58.7 ± 8.7	67.3 ± 8.1	5.0 ± 0.0***	62.2 ± 8.3
V. albo-atrum	9.9 ± 1.1***	5.0 ± 0.0***	7.0 ± 0.6***	7.7 ± 0.7***	5.0 ± ±0.0***	5.0 ± 0.0***	5.4 ± 0.1***	5.0 ± 0.0***	5.0 ± 0.0***	10.4 ± 1.4***	10.9 ± 1.4***	6.6 ± 0.5***	16.2 ± ±1.8
V. tenerum	12.2 ± 1.8	5.0 ± 0.0***	7.1 ± 0.6**	5.2 ± 0.1***	5.0 ± 0.0***	5.0 ± 0.0***	7.8 ± 0.8*	5.0 ± 0.0***	5.0 ± 0.0***	17.3 ± 2.6***	5.0 ± 0.0***	5.0 ± 0.0***	10.3 ± 1.2

[a] The 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 $p < 0.001$.

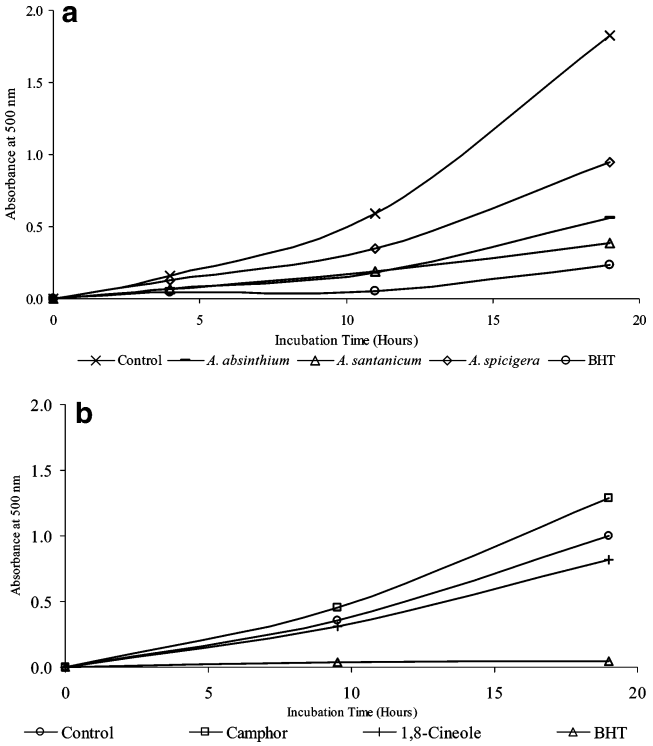

Figure 1. Comparison of antioxidant activities of (**a**) essential oils (100 μg/mL) and BHT (25 μg/mL) and of (**b**) commercially obtained camphor, 1,8-cineole (100 μg/mL), and BHT (50 μg/mL).

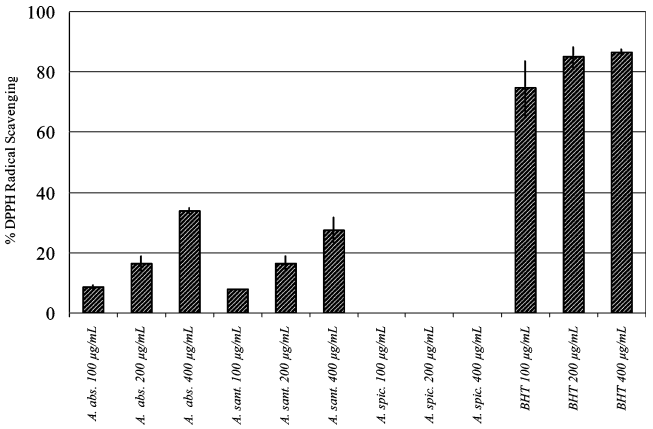

Figure 2. Comparison of DPPH scavenging activities of essential oils and BHT at 100, 200, and 400 μg/mL concentrations.

concentration (**Figure 1b**) nor DPPH radical scavenging activity at 100, 200, and 400 μg/mL concentrations (result is not shown). These results indicate that there is no clear relationship between major components and activities. Thus, the antioxidant activity of *A. absinthium* and *A. santonicum* oils could be attributed to other major and/or minor components. The antioxidant activity of *A. spicigera* oil was lower than that of *A. santonicum* and *A. absinthium*. On the other hand, BHT showed higher antioxidant activity as compared with essential oils of *Artemisia* species studied **(Figure 1a)**.

DPPH radical scavenging activities of essential oils increase with increasing amount of essential oils. The highest activity was found in *A. absinthium* oil among the essential oils studied **(Figure 2)**. Although the activity of *A. santonicum* oil was very close to the activity of *A. absinthium*, there was no detectable DPPH radical scavenging activity in *A. spicigera* oil. Nevertheless, the oils of *A. santonicum* and *A. spicigera* are similar in terms of most major volatile components. Therefore, it can be

concluded that there is no clear correlation between DPPH radical scavenging activities and the major components of essential oils.

The essential oils isolated from *Artemisia* species studied consist of various components **(Table 1)**. Therefore, determination of the component(s) responsible for activity is very difficult. Phenolic compounds such as thymol and carvacrol and essential oils rich in phenolic compounds show the potent antioxidant and DPPH radical scavenging activities (*35*, *36*). Thymol and carvacrol were detected in low amounts in all essential oils of *Artemisia* (**Table 1**). However, some researchers show that some essential oils rich in nonphenolic compounds also have antioxidant potentials (*19*, *28*, *29*). **Table 1** shows that essential oils of *Artemisia* are markedly rich in nonphenolic components. Because of this, activities of *Artemisia* essential oils can be attributed to nonphenolic constituents.

The *Artemisia* oils have a characteristic flavor, due to the presence of many components with strong sensory properties at low concentration. Some *Artemisia* species that do not contain thujone derivatives are used in the liqueur-making industry. Consequently, essential oils of *A. santonicum* and *A. spicigera*, which do not contain thujone derivatives, may be used in the liqueur-making industry because of its moderate antioxidant activities.

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Received for review September 20, 2004. Revised manuscript received December 15, 2004. Accepted December 20, 2004. We are indebted to Ataturk University for financial support of this work and to the State Planning Organization of Turkey (DPT) for purchasing a GC-MS instrument.

JF048429N