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Chemical Analysis and Biological Activities of Essential Oils from Trunk-Barks of Eight Trees

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The present study was conducted to evaluate chemical compositions and antimicrobial and antioxidant activities of essential oils from trunk barks of *Eucalyptus globulus, Juniperus oxycedrus* L, *Pinus nigra, Cedrus libani* A. Rich, *Abies equi-trojani, Cupressus sempervirens, Juglans regia* and *Alnus glutinosa* Mill. The chemical composition of hydro-distilled essential oils of the 8 trunk-bark samples was analyzed by gas chromatography-mass spectrometry. *E. globulus, J. oxycedrus, P. nigra, C. libani, A. equi-trojani, C. sempervirens, J. regia* and *A. glutinosa* 56, 52, 34, 56, 57, 46, 13 and 14 components were identified in the essential oils of the plants mentioned above, respectively. Antioxidant activities were measured employing free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability of the samples. All essential oils tested showed moderate free radical scavenging activity. The essential oil of *C. sempervirens* showed the highest scavenging activity (SC_{50}: 70 μg mL^{-1}) while that of *A. equi-trojani* showed the lowest (SC_{50}: 5480 μg mL^{-1}). The antimicrobial activity was studied by the agar diffusion method using 5 bacteria and a yeast-like fungus. The essential oils of the 8 species extended significant activity against *C. tropicalis*. The essential oil of *C. libani* was particularly active against the 5 bacteria studied with an minimal inhibitory concentration (MIC) at 250 μg mL^{-1} inactive.

Key Words: Trunk-bark, Antioxidant, Antimicrobial, Free radical scavenging, Essential oil.

INTRODUCTION

The essential oils, also known as volatile or etheric oils are aromatic oily liquids obtained from plant materials like flowers, buds, seeds, leaves, twigs, bark, wood, fruit and roots[1,2]. They can be obtained by expression, fermentation, effleurage or extraction, but steam distillation is the most commonly used one for commercial production of essential oils[2]. Essential oils are mostly natural mixtures of terpenes

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and terpenoids. Most of which are obtained from aromatic and pharmaceutical plants. The essential oils are used as functional ingredients in food, drinks, cosmetics and acaricidal preparations[3,4].

Plants have attracted scientists from many different fields for their biological and medicinal properties. The parts of the plants employed are the roots, stems, leaves, fruit and flowers. In recent years, the extracts from the barks of trees have also been popular[5-8]. Some trunk-bark extracts being were used as analgesic, anti-inflammatory, antibacterial, antiseptic and antifungal medicines[9]. Although volatile constituents of the leaves, needles, berries and flowers of *Abies equi-trojani*[9], *Cupressus sempervirens*[10], *Eucalyptus globulus*[10], *Juniperus oxycedrus*[11-13], *Pinus nigra*[14] and *Cedrus libani*[15] are available in the literature, but there is no comprehensive investigation have been credited for the essential oils obtained from the trunk-barks of these species.

The present paper, reports the antioxidative and antimicrobial capacities of the essential oils from the trunk-barks of 8 species with their chemical compositions.

EXPERIMENTAL

The trunk-bark samples of *J. oxycedrus*, *C. sempervirens*, *P. nigra*, *A. glutinosa*, *J. regia* and *E. globulus* were collected from Black sea region of Turkey. A. equi-trojani was collected from Mediterrinean Region of Turkey and *C. libani* was collected from Taurus Mountains in Antalya.

Isolation procedure: The essential oil of trunk bark powders (25 g) was obtained by hydro-distillation by using a Clevenger-type apparatus for 3 h, with the yields the whole of barks was *ca.* 0.25 %. The essential oils were taken by dissolving in HPLC-grade *n*-hexane (0.5 mL) and kept at 4 °C in a sealed brown vial until tested.

Gas chromatography-mass spectrometry (GC-MS): GC-MS analyses were performed by using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used as the detector. The chromatographic column used for the analyses was HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL s^{-1}. The injections were performed in splitless mode at 230 °C. One μL essential oil solution in hexane was injected and analyzed with the column held initially at 60 °C for 2 min, then increased to 260 °C with a 5 °C heating ramp and subsequently kept at 260 °C for 13 min. The relative percentage amounts of the separated compounds were calculated from total ion chromatograms by a computerized integrator. The identification of separated components was based on GC retention index (RI) values calculated by using the retention times of the sample components and those of standard alkanes[16,17].

Antioxidant activity: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the essential oil solutions in hexane was measured by the method of Yu *et al.*[18] with a slight modification. Briefly, 750 μL of sample solution of various concentrations (0.3, 0.15, 0.075, 0.0375 and 0.01875 mg mL^{-1} in hexane) was added to 750 μL of 50 μM ethanolic DPPH solution. Following a 50 min incubation period

in an ice bath, absorbance was read at 517 nm. Two different blanks were used and solvent blank being a mixture of hexane-ethanol (1:1) and sample-blank containing 750 µL sample solution and 750 µL ethanol. Butylated hydroxytoluene (BHT) and quercetin, both stable antioxidants, were used as synthetic references. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. SC_{50} (µg mL^{-1}), the antioxidant concentration to achieve 50 % radical scavenging, which was calculated from the curves drawn by plotting absorbance values for corresponding sample concentrations, was used to evaluate radical scavenging activities of the samples. All of the experimental results are presented as mean ± SD of triplicate measurements.

Identification of the compounds with the GC-MS method was made by a typical library search (National Institute of Standards and Technology and Wiley libraries) and with mass spectra literature data[19].

Antimicrobial activity: All test microorganisms, 6 bacteria and a yeast-like fungus, were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follow: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 702 ROMA and *Candida tropicalis* ATCC 60193. The essential oils from the bark samples were dissolved in hexane to prepare sample stock solutions of 1000 µg mL^{-1}.

Using a modification of the assay described by Southwell *et al.*[20], essential oil solutions were added to molten Mueller Hinton Agar (MHA) and Potato dextrose agar (PDA)/Tween-20 medium at 48 ºC, to give concentrations ranging from 4 to 500 µg mL^{-1}. The antibacterial and antifungal assays were performed in MHA (Difco, Detroit, MI) at pH 7.3 containing 1 % agar and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0 with 1 % agar, respectively. Plates prepared in triplicate were spot inoculated with 3 µL aliquots of culture in Mueller Hinton Broth (MHB) adjusted to yield a density within McFarland 0.5 turbidity. Plates were incubated at 37 ºC for 18 h and the minimal inhibitory concentration (MIC) was determined as the lowest concentration of the sample resulting in no growth of the inoculums on 2 of 3 plates. The essential oils were dissolved in chloroform to prepare stock solutions. Hexane and methanol-dimethyl sulphoxide (4:1) were used as control. Ampicillin and fluconazole were the standard drugs used as reference.

RESULTS AND DISCUSSION

The essential oils with a pale brown colour and cinnamon-like odour were obtained by hydro-distillation in a Clevenger-type apparatus (Ildam, Ankara, Turkey) from the trunk-bark tissues of the 8 samples. Table-1 shows scientific, family, local and common names of the 8 tree species and essential oil yields from their trunk barks. All the investigated trunk-bark samples contain essential oils that ranged from 0.11 to 0. 52 % based on dry weight (Table-1). The highest oil contents were found in *Juniperus oxycedrus* (0.52 %) and *Eucalyptus globules* (0.34 %).

TABLE-1
FAMILY NAMES, LATIN NAMES, COMMON NAMES, TURKISH (LOCAL) NAMES AND ESSENTIAL OIL YIELDS OF TRUNK-BARKS

Family	Latin name	Common name	Local name	Yield (%)
Myrtaceae	*Eucalyptus globulus*	Eucalyptus	Ökaliptus	0.34
Cupressaceae	*Juniperus oxycedrus*	Juniper	Ardiç	0.52
Pinaceae	*Pinus nigra*	Pine	Karaçam	0.21
Pinaceae	*Cedrus libani* A. Rich	Cedar	Lübnan sediri	0.12
Pinaceae	*Abies equi-trojani*	Fir	Kazdagi göknari	0.14
Cupressaceae	*Cupressus sempervirens*	Cypress	Servi	0.19
Juglandaceae	*Juglans regia*	Walnut	Ceviz	0.13
Betulaceae	*Alnus glutinosa* Mill.	Alnus	Kizilagaç	0.11

The essential oils obtained were analyzed by GC-MS with HP-5 column. The main constituents of the essential oils, the percentage of the components and their RI are summarized in Table-2. GC-MS analysis of the essential oil samples yielded the identification of the components from *E. globulus* (79.6 %), *J. oxycedrus* (90.6 %), *P. nigra* (59 %), *C. libani* (68.3 %), *A. equi-trojani* (59.6 %), *C. sempervirens* (34.7 %), *J. regia* (77.1 %) and *A. glutinosa* (56.4 %). Globulol (24.0 %), ferruginol (24.0 %), *p*-xylene (3.6 %), manool (11.0 %), α-terpineol (5.4 %), α-pinene (6.7 %), epiglobulol (36 %) and dibutylphthalate (8.0 %) were the main constituents of the essential oil of *E. globulus, J. oxycedrus, P. nigra, C. libani, A. equi-trojani, C. sempervirens, J. regia* and *A. glutinosa*, respectively. According to previous reports α-pinene (65 %) was the main component of berries and leaves of *J. oxycedrus* but, its amount changed with seasonal conditions[11]. The volatile constituents of leaf and berries of *J. oxycedrus* were in accordance with present results.

The chemical class distribution of the essential oil components of the plants are given in Table-3. The compounds were separated into seven classes, as terpenes, aromatic hydrocarbons, aliphatic hydrocarbons, alcohols, carboxylic acids and their esters and others.

Similarly, the main constituents of *C. sempervirens* and *E. globules* were determined as sabinen (39 %) and α-pinene (20 %), respectively by Saccahetti *et al.*[10].

It was found that the major component in *Cedrus libani* was manool (11 %). Baser *et al.*[15] were characterized 37 constituents in the essential oils from the wood and root parts of *C. libani*. The finding was similar to present results, but no report was available in the literature, concerning the chemical constituents of the essential oils of *A. glutinosa*. The main component of *A. glutinosa* was dibutylphthalate (8.0 %) and linoleic acid (7.7 %).

Several methods have been employed to determine antioxidant activity of biological samples and the results are compared with those of reference antioxidant standards in various investigations[21]. DPPH is long-lived nitrogen radical and suitable to measure antioxidant capacity of essential oils, which are non-polar fractions[22]. In the present study, the free radical scavenging activity of the essential oils was

TABLE-2
MAIN COMPONENTS OF ESSENTIAL OILS FROM
THE TRUNK-BARKS OF EIGHT SPECIES

Compound[a]	A	B	C	D	E	F	G	H	RI[b]	Ref.
Phenylethane	–	–	0.5	0.2	–	tr	0.4	–	792	19
p-Xylene	0.10	tr	3.6	0.6	tr	0.1	4.1	4.3	795	19
Santen	–	–	–	–	0.4	–	–	–	905	19
1,3-Dimethylbenzene	tr	–	0.9	0.4	–	0.1	–	–	912	19
Tricyclene	–	0.2	–	–	0.1	–	–	–	934	17
Thujene	–	–	–	–	–	1.2	–	–	937	17
α-Pinene	0.7	1.0	0.2	0.1	1.7	6.7	–	–	943	17
Camphene	0.1	0.3	0.4	2.4	1.4	0.7	–	–	953	17
Verbenene	–	0.4	–	–	1.1	tr	–	–	957	7
Benzaldehyde	–	–	–	–	0.1		–	–	962	17
Sabinene	–	–	–	–	–	3.2	–	–	971	17
β-Myrcene	0.1	–	–	–	–	2.2	–	–	983	17
2-Amylfuran	–	–	–	–	–	–	2.4	7.5	983	8
1,5,8-*p*-Menthatriene	–	0.2	–	–	–	–	–	–	992	8
α-Phellandrene	1.1	–	–	–	–	–	–	–	992	10
3-Carene	–	–	–	–	tr	0.1	–	–	995	11
Isocineole	–	0. 1	–	0.2	–	–	–	–	998	8
o-Cymol	0.9	0.3	0.2	0.8	–	–	–	–	1105	8
Cinen	–	–	–		0.9	–	–	–	1108	8
Terpan	7.0		–	0.4	–	–	–	–	1109	8
Moslene	Tr	0.1	–	0.1	tr	0.1	–	–	1127	8
Limonene	–	0.1	–	–	–	–	–	–	1030	8
Dehydro-p-cymene	0.4	–	–	2.5	0.5		–	–	1144	19
p-Isopropenyl toluene	–	–	–	–	–	0.2	–	–	1145	19
α-dimethylstyrene	–	1.1	–	–	–	–	–	–	1145	19
Fenchol	tr	0.1	0.4	0.7	0.8	tr	–	–	1158	19
α-Campholene aldehyde	tr	0.7	–	–	–	–	–	–	1164	17
3-Cyclopentene-1-acetaldehyde	–	–	0.1	–	0.2	0.1	–	–	1164	8
L-Pinocarveol	–	1.1	0.2	0.9	–	0.2	–	–	1171	17
2-Bornanone	–	–	1.2	–	–	–	–	–	1173	8
exo-Methyl-camphenilol	–	–	–	–	0.3	–	–	–	1176	8
Pinocarvone	–	0.7	–	–		0.2	–	–	1182	8
Borneol L	0.3	–	1.1	2. 0	1.6	–	–	–	1184	2
trans-p-Menth-2-ene-1,8-diol	–	1.2	–	–	–	–	–	–	1184	17
Cyclopentane,1,2-dimethyl-3	0.1	0.1	0.8	–	–	–	–	–	1187	8
4-Terpineol	0.2	0.9	–	0.7	3.8	0.4	–	–	1189	17
3-Methylacetophenone	0.1	–	0.2	0.5	–	–	–	–	1192	8
para-Cymen-8-ol	–	–	–	–	0.2	0.1	–	–	1193	10
α-Terpineol	0.4	–	0.3	1.2	–	–	–	–	1195	17
Linalyl propionate	–	0.4	–	–	–	–	–	–	1195	8
Benihinal	–	–	–	–	–	0.1	–	–	1197	8
Bicyclo[3.1.1]hept-2-ene-2-ca	–	1.9	0.4	0.2	0.9	0.1	–	–	1197	8
Myrtenal	–	–	–	0.7	0.9	–	–		1197	17
Sabinol	0.1	–	–	–	–	–	–		1200	19
Berbenone	tr	–	0.5	2.3	–	–	–	–	1207	10
Verbenone	–	–	–	–	–	0.2	–	–	1207	17
t*rans-(+)*-Carveol	0.1	0.2	–	0.2	0.1	tr	–	–	1219	17
Thymyl methyl ether	–	–	–	0.1	–	–	–	–	1236	17

Compound[a]	A	B	C	D	E	F	G	H	RI[b]	Ref.
2-Methyl-3-phenylpropanal	0.1	–	–	0.1	–	–	–	–	1240	19
(+)-Carvotanacetone	0.3	–	–		–	–	–	–	1248	17
Piperitone	0.3	–	–	0.1	–	–	–	–	1255	17
5-Pentyl-3*H*-furan-2-one	–	–	–	–	–	–	0.7	–	1266	17
Pelargon	–	0.1	0.6	–	–	–	–	–	1273	17
Perillal	0.1	–	–	–	–	–	–	–	1274	17
1-Cyclohexene-1-carboxaldehyde	–	–	–	–	0.1	–	–	–	1275	19
Nonanoic acid	0.1	–	–	–	–	–	–	–	1279	17
Anethole	–	tr	–	0.2	0.1	–	–	–	1286	17
Bornyl acetate	–	0.1	–	–		–	–	–	1286	17
Cuminol	–	–	–	–	0.1	–	–	–	1291	19
α-Campholenic acid	–	0.1	–	–	–	–	–	–	1294	19
Thymol	0.1	–	–	–	–	–	–	–	1294	17
Antioxine	–	–	–	–	tr	0.1	–	–	1302	19
Carvacrol	0.3	0.6	–	0.1	tr	0.2	–	–	1302	17
p-Vinylguaiacol	–	–	–	–	0.1	0.1	–	–	1313	19
Triethylbenzene	0.1	–	–	–	–	–	–	–	1327	19
2-Methylenebornane	–	–	–	–	0.1	–	–	–	1341	19
Cyclofenchene	–	–	–	–	–	0.1	–	–	1341	19
exo-2-Hydroxycineole acetate	0.1	–	–	–	–	–	–	–	1343	19
(+)-2-Carene	1.0	–	–	–	–	–	–	–	1351	19
α-Cubebene	–	–	–	–	–	0.8	–	–	1351	17
4-Pentylbutan-4-olide	–	–	–	–	–	–	1.3	–	1363	19
Longicyclene	–	–	–	0.3	0.2	0.1	–	–	1371	17
Copaen	–	–	–	0.4	0.1	0.2	–	–	1377	17
Germacrene-D	–	–	–	–	–	0.3	–	–	1391	19
β-Elemene	–	–	–	–	0.1	–	–	–	1392	17
Jasmone	–	–	–	–	0.1	–	–	–	1398	17
Methyleugenol	–	–	–	–	0.1	–	–	–	1404	17
Isolongifolene	–	–	–	9.7	–	–	–	–	1405	17
α-Cedrene	–	0.2	–	–	–	–	–	–	1407	1
α-Gurjunene	0.7	–	–	–	–	–	–	–	1410	17
Cedrylmethylether	–	–	–	–	–	0.7	–	–	1413	8
Caryophyllene	–	0.5	–	–	0.1		–	–	1420	17
γ-Muurolen	–	–	–	–	–	0.5	–	–	1421	8
Thujopsene	–	–	–	–	–	0.1	–	–	1432	17
Aromadendrene	9.7	0.3	0.8	0.2	–	–	–	1.8	1440	19
Humulene	–	0.1	–	–	–	–	–	–	1455	8
Cadina-1,4-diene	–	–	–	–	–	0.1	–	–	1465	8
Ethyl cinnamate	–	–	–	0.2	–	–	–	–	1466	17
Longifolene	0.9	–	–	–	–	4.7	–	–	1489	8
(+)-Epi-bicyclosesquiphell	–	–	–	–	–	0.2	–	–	1492	8
α-Terpineol	–	–	–	–	5.4	–	–	–	1495	8
α-Selinene	–	0.1	–	–	–	0.1	–	–	1495	8
Viridiflorene	1.4	tr	–	–	–	–	–	–	1496	17
α-Muurolene	–	–	–	–	1.0	–	–	–	1500	17
Buks	–	–	0.8	–	–	–	–	–	1513	8
N-*t*-Butyl-N'-(diisopropylphosphinoylhydrazine)	–	–	–	–	–	–	1.9	–	1517	8
cis-Calamenene	0.3	0.2	–	1.2	0.4	–	–	–	1525	17
Δ-Cadinene	–	–	–	0.4	–	1.5	–	–	1525	17
Valencene	1.2	–	–	–	–	–	–	–	1540	8

Compound[a]	A	B	C	D	E	F	G	H	RI[b]	Ref.
2*H*-Benzocyclohepten-2-on	0.2	–	–	–	–	–	–	–	1549	8
Germacrene	0.2	–	–	–	–	–	–	–	1555	8
Longicamphenylone	–	–	–	2.8	–	0.2	–	–	1563	8
Epiglobulol	6.0	0.7	–	–	–	–	36	–	1564	17
α-Calacorene	–	–	–	–	0.1	–	–	–	1565	17
Selina-3,7(11)-diene	2.3	–	–	–	–	–	–	–	1570	8
Globulol	24.0	–	–	–	–	–	–	–	1591	10
7-Amino-1,4-dimethylpyrim	–	–	–	–	–	0.3	–	–	1591	8
Salvial-4 (14)-en-1-one	–	–	–	–	0.2	–	–	–	1594	17
Longiborneol	–	–	–	1.0	–	–	–	–	1597	17
α-Patchoulene	–	–	–	1.3	–	–	–	–	1605	19
Cedrol	–	2.3	–		–	6.0	–	–	1605	17
8-β-H-Cedran-8-ol	–	–	–	–	–	0.3	–	–	1615	19
2,5-Diethyl-3,6-dimethylpy	1.7	–	–	–	–	–	–	–	1626	19
α-Elemene	–	–	–	–	–	–	–	1.6	1631	19
Azulene	2.0	–	–	–	–	–	–		1632	19
Calarene β-gurj	–	–	–	–	–	–	13	–	1632	19
Caryophyllen	–	4.9	–	0.6	–	0.2	–	–	1638	17
Hinesol	–		–	–	–	–	12	–	1641	17
Valencene	–	0.2	–	–	–	–	–	–	1644	19
α-Cadinol	3.0	–	–	0.7	–	0.5	–	–	1644	17
Torreyol	–	–	–	–	–	0.5	–	–	1650	17
Vulgarone B	–	–	–	1.7	–	–	–	–	1651	17
2-Naphthalenemethanol	1.8	–	–	–	–	–	–	4.4	1652	19
β-Selinenol	–	–	–	–	–	–	–	–	1652	19
Junipen	–	–	–	–	–	0.6	–	–	1675	19
Impurity	–	–	–	1.3	–	–	–	–	1701	17
7-Methoxy-4-methylcarboxilic acid	–	–	–	–	0.2		–	–	1729	19
Isoaromadendrene epoxide	–	–	–	–	–	0.2	–	–	1739	19
Ambrox	–	–	–	–	0.3	–	–	–	1757	17
Calarene	0.72	–	–	–	0.1	–	–	–	1763	19
Tetradecanoic acid	–	0.4	0.4	–	–	–	–	–	1763	19
Aristolone	0.57	–	–	–	–	–	–	–	1798	19
E-2-Tetradecen-1-ol	–	0.8	0.8	–	–	–	–	–	1817	19
Podocephalol	–	–	–	0.5	–	0.1	–	–	1840	19
Palatinol IC	–	–	–	–	–	–	0.8	4.0	1869	19
Cetene	–	3.0	1.8	–	–	–	–	–	1880	19
Chromolaenin	–	–	–	0.3	–	–	–	–	1892	19
Pentadecanoic acid	–	–	–	–	0.2	–	–	–	1897	19
9-Cedranone	–	–	–	–	–	–	–	1.4	1919	19
Metholene	–	0.1	–	–	0.6	–	–	–	1928	19
7-ethenyl-1-phenanthrene	–	–	–	0.2	0.2	–	–	–	1939	19
15,16-Dinorlabd-8(20)-en-13-one	–	–	–	0.7	–	–	–	–	1954	19
N-Acetyl-3,4-methyleneddioxymethamphet-amine	0.18	–	–	–	–	–	–	–	1957	19
Dibutyl phthalate	–	–	–	–	–	–	0.7	8.0	1963	19
Sandaracopimaradiene	–	–	–	–	0.2	–	–	–	1964	19
n-Hexadecanoic acid	0.70	0.7	0.7	–	2.2	–	–	–	1967	19
p-Dimethylamino benzyli	–	–	–	0.4		–	–	–	1968	19
Epimanoyl oxide	–	–	0.1	1.8	1.0	–	–	–	1992	19
Manoyl oxide	–	–	–	–	4. 0	–	–	–	1993	19

Compound[a]	A	B	C	D	E	F	G	H	RI[b]	Ref.
8-Dimethyl-2-isopropyphenanthrene octahydrone	–	–	–	–	1.3	–	–	–	2017	19
Δ-10-Dehydroepijuvabione	–	–	–	1.6	–	–	–	–	2043	19
6,8,9-Trimethoxy-3-methythenzo[9] isoquinoline-5,10-quinone	–	0.3	0.3	–	–	–	–	–	2052	8
Abietate	–	–	–	3.1	–	–	0.6	–	2057	11
7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10a-octahydrophenanthrene	–	–	–	–	2.8	–	–	–	2058	19
Manool	–	–	–	11.0	–	–	–	–	2058	17
α-Farnesene	0.1	–	–	–	–	–	–	–	2061	19
1-Chloro-octadecane	–	0.9	1.0	–	–	–	–	–	2100	19
Methyl oleate	–	–	–	–	2.6	–	–	–	2102	19
Octadec-9-en-18-olide	–	7.1	4.7	–	–	–	–	–	2146	19
Linoleic acid	0.3	–	–	0.6	2.6	–	–	7.7	2146	19
Octadecadienoic acid	–	–	–	–	3.2	–	–	–	2147	19
1-Allyl-3-methylindole-2-carbaldehyde	0.5	2.1	4.9	0.4	–	–	–	–	2182	19
Docosane	0.5	3.4	8.0	–	–	–	–	–	2194	17
10,11-(4,5'dimethylbenzo)[3,2] *p*-cylophane	–	17	–	–	–	–	–	–	2212	19
9,0-Dihydro-9-methoxy-9,10	–	–	–	1.1	–	–	–	–	2219	19
1-(1,1-Dimethyl-6-indanyl)4,4-phenyl-2-butyne-1-4-dione	–	0.7	–	–	–	–	–	–	2232	19
1,7-Dimethyl-2-oxo-7-(4'-formyl-buthyl)-norbornane	–	–	1.0	–	–	–	–	–	2232	19
5-Hydroxy-1,3,4-trimethoxy-7-methyl-6-proparegygynaphtane	–	–	–	0.3	–	–	–	–	2244	19
12-(Cyanomethyl) indolo [1,2] quinazoline	–	–	3.7		–	–	–	–	2252	19
Dehydroabietal	–	–	–	0.6	3.9	–	–	3.8	2259	17
Dehydroabietic acid	tr	–	2.4	0.7	2.8	–	–	–	2271	19
Abietadien	–	–	–	–	4.2	–	–	–	2280	11
Tricosane (CAS)	–	–	2.4	–	–	–	–	2.0	2301	17
Totarol	–	6.5	–	–	–	–	3.2	3.4	2309	17
Ferruginol	–	24.0	–	–	–	–	–	–	2332	17
2-Dodecen1ylsuccinic anhydrate	–	–	0.5	–	–	–	–	–	2365	19
7-Isopropyl-4-[2'-(4'-methoxyp	–	0.5	–	–	–	–	–	–	2365	19
4-Epiabietol, dehydro	–	–	–	1.1	–	–	–	–	2366	19
2-(4-Bromophenyl)-5,6-dihydr	–	–	–	0.4	–	–	–	–	2381	19
Abietic acid methyester	–	–	–	–	1.0	–	–	–	2387	19
12-β-hydroxypicras-4-en-3-	–	0.7	–	–	–	–	–	–	2390	18
Oleic acid	–	–	–	–	1.9	–	–	–	2398	19
Tetracosane	1.2	–	2.8	–	–	–	–	2.7	2401	17
Tetramethylspiro[4.4]nonane	–	–	0.7	–	–	–	–	–	2431	19
Labda-8(17),13(E)-diene-15-ol	–	–	tr	0.8	–	–	–	–	2489	19
2-Methyl-3,13-octadecadienol	–	–	–	–	0.5	–	–	–	2495	19
Pentacosane	–	–	0.2	–	–	–	–	3.8	2501	17
Podocarpa-8,11,13-trien-3-one,	–	0.9	–	–	–	–	–	–	2514	19
Koiganal II	–	–	–	–	0.4	–	–	–	2549	19
Hahnfett	–	–	0.4	–	–	–	–	–	2565	19
14-Hydroxytaxodione	–	0.6	–	–	–	–	–	–	2570	19
7-Pentadecyne	–	–	–	–	0.3	–	–	–	2591	19
Hexacosane	0.6	–	3.1	–	–	–	–	–	2601	19
Heptacosane	–	–	2.2	–	–	–	–	–	2701	19
1-Chloro-nonadecane	–	0.1	–	0.1	–	–	–	–	2701	19

Compound[a]	A	B	C	D	E	F	G	H	RI[b]	Ref.
Octacosane (CAS)	0.3	–	1.7	–	–	–	–	–	2802	19
Eicosane (CAS)	–	0.2	0.7	0.1	–	–	–	–	2862	19
Total	79.6	90.6	59	68.3	59.6	34.7	77.1	56.4	–	–

A = *E. globulus*, B = *J. oxycedrus*, C = *P. nigra*, D = *C. libani*, E = *A. equi-trojani*, F = *C. sempervirens*, G = *J. regia*, H = *A. glutonisa*
[a]Compounds listed in order of elution from a HP-5 column.
[b]Kovats Index on HP-5 column in reference to *n*-alkanes.
[c]tr; trace < 0.06 %.

TABLE-3
CHEMICAL CLASS DISTRIBUTION OF THE ESSENTIAL OILS FROM THE EIGHT TRUNK-BARKS

Compounds class	A	B	C	D	E	F	G	H
Terpenes	69.7	59.0	10.9	53.5	37.6	28.1	69.9	22.5
Aromatic hydrocarbons	2.2	1.5	4.1	1.7	-	0.4	1.0	11.0
Aliphatic hydrocarbons	0.7	5.6	11.4	1.2	0.5	-	-	8.5
Aldehydes	1.0	7.8	9.6	0.6	0.5	0.1	-	-
Alcohols	0.9	1.1	2.1	1.2	1.7	0.2	4.1	4.4
Carboxylic acids and their esters	1.1	1.2	6.0	1.6	12.8	0.6	-	8.1
Others	4.1	14.4	13	8.5	4.8	5.3	2.0	2.9
Total	79.7	90. 6	59	68.3	59.6	34.7	77.1	56.4

A = *E. globulus*, B = *J. oxycedrus*, C = *P. nigra*, D = *C. libani*, E = *A. equi-trojani*, F = *C. sempervirens*, G = *J. regia*, H = *A. glutonisa*

assayed by using the known antioxidants BHT and quercetin with employing *in vitro* DPPH assay. The results yielded a concentration dependent pattern. The essential oils of *C. sempervirens*, *J. oxycedrus*, *E. globules* and *C. libani* showed remarkably high DPPH radical scavenging activity (Fig. 1). The scavenging activities of the samples were lower than those of the positive controls and better than those reported

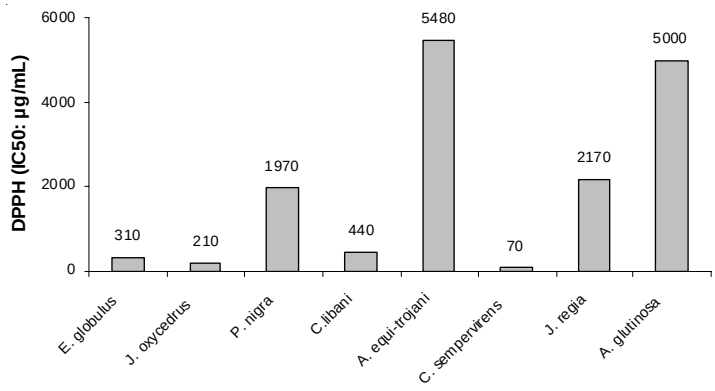

Fig. 1. DPPH radical scavenging activity (SC_{50}: µg mL^{-1}) of the essential oils of eight trunk bark species from Turkey. Standard antioxidants used were BHT (SC_{50}: 9.8 ± 0.3 µg mL^{-1}) and quercetin (SC_{50}: 2.5 ± 0.1 µg mL^{-1})

by Sacchetti *et al.*[10]. The research data about the free radical scavenging capacity of essential oils clearly show that the methods and results in many stages of antioxidant activity measurements vary remarkably and it is difficult to compare the results of one investigation with another.

The antimicrobial activity was studied by measuring the MIC using 5 bacteria and a yeast-like fungus (Table-4). The oil from *C. libani* showed antimicrobial activity against the 5 bacteria at 250 μg mL^{-1} concentration. In addition, all the samples showed high antifungal activity against *C. tropicalis*. Yesilada *et al.*[22] reported the methanolic and aqueous extracts of *C. libani* to have antibacterial effect against *H. pylori*. In addition, Sacchetti *et al.*[10] reported that the essential oil of *C. sempervirens* inhibited the growth of pathogenic yeasts *C. albicans, R. glutinosa* and *S. cerevisiae*. These finding are also in accordance with the results given here.

TABLE-4
SCREENING FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL (MIC 4-500 μg mL^{-1})

Samples	Microorganisms and MIC value						
	Ec	Kp	Pa	Ef	Sa	Bc	Ct
E. globulus	–	–	–	–	–	–	16
J. oxycedrus	–	–	–	–	–	–	16
P. nigra	–	–	–	–	–	250	16
C. libani	250	250	250	250	250	250	62
A. subbps.	–	–	–	–	–	–	32
C. sempervirens	–	–	–	–	–	–	32
J. regia	–	–	–	–	–	–	32
A. glutinosa Mill.	–	–	–	–	–	–	32
R. ponticum	250	250	250	250	250	250	62
Amp.	8	32	>128	2	2	2	
Flu.							8

Ec: *Escherichia coli* ATCC 25922, Kp: *Klebsiella pneumoniae* ATCC 13883, Pa: *Pseudomonas aeruginosa* ATCC 10145, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bc: *Bacillus cereus 702 Roma*, Ct: *Candida tropicalis* ATCC 60193. Amp.: Ampicillin, Flu.: Fluconazole, (–): no activity (250-500 μg mL^{-1}).

To conclude, the present study is a contribution to the better knowledge of the chemical analysis and biological activities of the essential oil from trunk-barks of 8 trees. However, it is obvious that further investigations are needed to elucidate the entire chemical composition and to determine the exact contribution of each component to the biological activities. This essential oil may be produced by local population for application in folk medicine and aromatherapy, possibly by commercial exploitation as sustainable development.

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