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Antimicrobial activity and volatile constituents of the flower, leaf, and stem of *Paeonia daurica* grown in Turkey

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The volatile constituents of the essential oils from the flower, leaf, and stem of *Paeonia daurica* Andrews were analyzed by GC and GC-MS. A total of 74 compounds were identified, constituting over 95.0%, 80.8%, and 98.2% of the oil composition of the flower, leaf, and stem of *P. daurica*, respectively. Aldehydes were shown to be the main group of constituents of the flower and stem parts, at 39.1% and 79.8%, respectively. However, the major group in the leaf oil was found to be oxygenated monoterpenes (43.5%). Salicylaldehyde (20.7% and 79.5%) was the major component of the flower and stem oils of *P. daurica*, respectively. Linalool (31.4%) was the main compound of the leaf oil. The antimicrobial activity of the isolated essential oils of the plant was also investigated, and they showed moderate antibacterial activity against tested microorganisms.

Key Words: *Paeonia daurica*, essential oil, antimicrobial activity, GC-MS

Introduction

The genus *Paeonia* L. (Paeoniaceae) contains a number of species, and among them, 12 taxa of *Paeonia* were recorded in Turkey.[1–3] They have large red flowers, and several species are used as ornamental plants.[4] The species of *Paeonia* are also known as 'the queens of herbs,' a title deserved on account of the beauty of their flowers and their medicinal properties. *Paeonia* species, and especially their roots, are among the the most

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important sources of crude drugs in traditional Chinese medicine.[5] They have been said to possess sedative, analgesic, and antiinflammatory properties, and they have been used as a remedy for cardiovascular diseases, female genital diseases, and eczema.[5−10] *P. mascula* (L.) Mill. and *P. peregrina* Mill. have been used as folk medicine against ulcers, coughs, and epilepsy in Anatolia.[11−12]

Despite their reported use for medicinal purposes, there have been only a few reports related to the chemical analysis of the volatile constituents of the roots of Chinese *Paeonia* species,[5,13] the Greek taxa, or the Turkish taxa.[14−17] The biological activities of *Paeonia* species have also been mentioned.[18−24] Essential oil compositions and antioxidant properties for the ethanol extract of the roots of *P. daurica* Andrews, which was collected from the western part of Turkey (Tepeköy, Mersin), have been reported,[14] and only 24 components were identified, as compared to 74 compounds in the present study. Salicylaldehyde was the major compound in both cases. In this report, GC-FID and GC/MS were used for the chemical analysis of the volatile constituents obtained by hydrodistillation of the flower, leaf, and stem of *P. daurica*. Antimicrobial activities against 3 gram-negative and 4 gram-positive bacteria, a mycobacterium, and 2 pathogenic fungi were evaluated.

Experimental

Plant material: *Paeonia daurica* was collected in Mesudiye, Ordu, stony places (altitude of approximately 1230 m), in the northeastern part of Turkey, 17 May 2009. The plant was authenticated by Prof. S. Terzioğlu.[1−3] A voucher specimen was deposited in the Herbarium of the Faculty of Forestry (KATO: 8410), Karadeniz Technical University, Turkey.

Isolation of the essential oils: The fresh plant materials were separated into flower, leaf, and stem parts and then ground into small pieces. The essential oils from fresh aerial parts (approximately 100 g each) of *P. daurica* were isolated by hydrodistillation in a Clevenger-type apparatus[9−12] with a cooling bath (-15 °C) system (4 h) (yields: 0.08%, 0.06%, and 0.12% (v/w), respectively). The obtained oils were extracted with HPLC grade n-hexane (0.5 mL), dried over anhydrous sodium sulfate, and stored at 4-6 °C in a sealed brown vial.

Gas chromatography: The capillary GC-FID analysis was performed using an Agilent-5973 network system, equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. The chromatographic column used for the analysis was an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness of 0.25 μm). Helium was used as a carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. With the column held initially at 60 °C for 2 min, 1 μL of essential oil solution in hexane (HPLC grade) was injected and analyzed, and then the temperature was increased to 240 °C with a 3 °C/min heating ramp. The identity of each compound was supported by comparing their retention index (RI) values, as found in the literature. The sample was analyzed twice and the percentage composition of oil was computed from the GC peak areas without using correction factors.

Gas chromatography-mass spectrometry: GC-MS analysis was performed 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. The chromatographic column used for the analysis was an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness of 0.25 μm). Helium was used as a carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. With the column held initially at 60 °C for 2 min,

1 μL of essential oil solution in hexane (HPLC grade) was injected and analyzed, and then the temperature was increased to 240 °C with a 3 °C/min heating ramp.

Identification of constituents: Retention indices of all of the components were determined by the Kovats method, using n-alkanes (C_6-C_{32}) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Wiley 7NL), authentic compounds (limonene, linalool, α-terpineol, geraniol, tridecane, tetradecane, pentadecane, nonadecane, eicosane, heneicosane, docosane, tricosane, tetracosane, and pentacosane), and data published in the literature.[25–29]

Antimicrobial activity assessment: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: Escherichia coli ATCC 25922, Yersinia pseudotuberculosis ATCC 911, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Listeria monocytogenes ATCC 43251, Bacillus cereus 702 ROMA, Mycobacterium smegmatis ATCC607, Candida albicans ATCC 60193, and Saccharomyces cerevisiae RSKK 251. All of the plant extracts were dissolved in diethyl ether to prepare extract stock solutions of 1000 μg/mL.

Agar-well diffusion method: A simple susceptibility screening test using the agar-well diffusion method[30] as adapted earlier[31,32] was used. Each bacterium was suspended in Mueller-Hinton (MH) broth (Difco, Detroit). The yeast-like fungi were suspended in yeast extract broths. The microorganisms were then diluted to approximately 10^6 cfu/mL. For yeast-like fungi, Sabouraud dextrose agar (SDA) (Difco) was used. They were "flood-inoculated" onto the surface of MH and SDA agars and then dried. From the agar, wells of 5 mL in diameter were cut using a sterile cork borer, and 50 μL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. *Mycobacterium smegmatis* was grown for 3-5 days on MHA plates at 35 °C.[33] Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 μg), streptomycin (10 μg), and fluconazole (5 μg) were the standard drugs. Diethyl ether was used as the control.

Results and discussion

The essential oil compositions from the flower, leaf, and stem of *P. daurica* are listed in Table 1. Altogether, 74 essential compounds were identified by GC and GC-MS with an HP-5 column from aerial parts of *P. daurica*. The flower oil revealed the presence of 41 components, representing 95.0% of the total oil. The major compounds of the flower oil were salicylaldehyde (20.7%), nonanal (17.1%), cyclopentadecanolide (7.7%), khusimone (7.5%), eugenol (6.8%), and germacrene D (4.6). In the leaf, 39 components were identified, representing 80.8% of the total oil. The major constituents of the leaf oil were linalool (31.4%), neryl formate (10.0%), α-terpineol (7.2%), nerol (4.3%), salicylaldehyde (3.4%), and $2E-$hexenyl benzoate (2.7%). From the stem oil, 15 components, accounting for 98.2% of constituents, were identified, and the major volatile constituents were salicylaldehyde (79.5%), methyl salicylate (5.1%), myrtenal (4.7%), linalool (1.7%), and heneicosane (1.3%).

The chemical class distributions of the essential oils of the constituents are summarized in Table 2. The compounds were separated into 5 classes, which were terpenoids (monoterpene hydrocarbons, oxygenated monoterpene, sesquiterpene hydrocarbons, oxygenated sesquiterpene, diterpenoid, and terpene-related compounds), esters, aldehydes, hydrocarbons, and others (Table 2). The aldehydes were the major constituents of the flower and stem parts of the plant, at 39.1% and 79.81%, respectively. The major component of the leaf oil

Table 1. Identified components in the essential oils of *P. daurica*.

Compounds	Ex. RI[a]	Lit. RI	Flower Area[b]	Leaf Area[b]	Stem Area[b]
Monoterpene hydrocarbons					
Limonene[c]	1029	1029	-	0.3	-
Z-β-Ocimene	1039	1037	-	0.1	-
Terpinolene	1090	1089	-	0.7	-
allo-Ocimene	1133	1132	-	0.2	-
Oxygenated monoterpenes					
Linalool[c]	1097	1097	-	31.4	1.7
α-Pinane oxide	1098	1099	-	0.3	-
α-Terpineol[c]	1190	1189	-	7.2	-
Myrtenal	1194	1196	-	0.3	4.7
Nerol	1233	1230	-	4.3	0.2
Geraniol[c]	1255	1253	0.6	-	-
E−Myrtanol	1261	1261	-	-	1.2
Perilla aldehyde	1273	1272	-	-	0.6
Sesquiterpene hydrocarbons					
β-Panasinsene	1380	1383	0.4	-	-
β-Elemene	1390	1391	0.2	-	-
Z-Caryophyllene	1412	1409	-	0.5	-
E-Caryophyllene	1420	1419	3.4	-	-
β-Copaene	1432	1432	0.4	-	-
α-Humulene	1454	1455	0.6	-	-
Germacrene D	1484	1485	4.6	-	-
Bicyclogermacrene	1498	1500	0.5	-	-
α-Muurolene	1499	1500	0.3	-	-
E, *E*- α-Farnesene	1508	1506	-	0.5	-
γ−Cadinene	1513	1514	0.4	-	-
δ-Cadinene	1522	1523	1.0	-	-
Oxygenated sesquiterpenes					
E−Nerolidol	1565	1563	0.7	-	-
Viridiflorol	1591	1593	0.7	-	-
epi-α-Cadinol	1639	1640	0.5	-	-
epi-α- Muurolol	1643	1642	0.7	-	-
α- Muurolol	1646	1646	0.3	-	-
α- Cadinol	1655	1654	0.9	-	-
Oxygenated diterpenes					
Phytol	1946	1943	-	1.0	-

Table 1. Continued.

Compounds	Ex. RI[a]	Lit. RI	Flower Area[b]	Leaf Area[b]	Stem Area[b]
Terpene-related compounds					
Neryl formate	1279	1282	-	10.0	-
Iso-dihydro carveol acetate	1331	1329	0.1	-	-
E-β-Damascenone	1384	1385	-	0.7	-
Geranyl acetone	1456	1455	0.5	-	-
E-β-Ionone	1490	1489	-	0.8	-
Khusimone	1607	1604	7.5	-	-
Hexahydrofarnesylacetone	1844	1847	0.6	0.1	-
Farnesyl acetone	1920	1920	1.0	0.1	-
Ester					
3*E*-Hexenyl acetate	1003	1002	-	1.5	-
3*Z*−Hexenyl acetate	1007	1005	-	1.4	-
Methyl salicylate	1196	1196	0.7	-	5.1
3*Z*-Hexenyl benzoate	1570	1567	0.3	1.5	-
Hexyl benzoate	1579	1580	-	0.3	-
2*E*-Hexenyl benzoate	1590	1588	-	2.7	-
Benzyl benzoate	1763	1760	-	0.2	-
Cyclopentadecanolide	1833	1834	7.7	-	-
Hexadecyl acetate	2005	2004	2.7	-	-
Aldehyde					
Salicylaldehyde	1046	1045	20.7	3.4	79.5
Nonanal	1102	1101	17.1	-	0.3
2*E*-Nonen-1-al	1165	1162	0.4	-	-
2E,4E-Decadienal	1320	1317	-	0.7	-
Dodecanal	1411	1409	0.9	-	-
Hydrocarbons					
Tridecene	1293	1292	-	-	0.4
Tridecane [c]	1300	1300	1.7	-	-
Tetradecene	1391	1389	-	0.8	-
Tetradecane[c]	1400	1400	-	0.4	-
Pentadecane[c]	1500	1500	-	0.4	-
Octadecene	1788	1790	-	0.5	-
Nonadecane[c]	1898	1900	0.4	0.2	-
Eicosane[c]	1999	2000	-	3.0	-
Heneicosane[c]	2099	2100	0.7	0.3	1.3
Docosane[c]	2200	2200	-	0.1	0.3
Tricosane[c]	2299	2300	0.9	0.8	0.3

Table 1. Continued.

Compounds	Ex. RI[a]	Lit. RI	Flower Area[b]	Leaf Area[b]	Stem Area[b]
Tetracosane[c]	2398	2400	0.2	0.4	-
Pentacosane[c]	2500	2500	1.2	0.3	0.2
Others					
Hepten-2-one	984	986	0.7	-	-
2-pentyl furan	991	993	-	0.4	-
Nopinone	1140	1140	-	0.3	2.0
2,3-Dimethyl benzofuran	1199	1196	-	2.7	0.4
Eugenol	1363	1359	6.8	-	-
n-Dodecanol	1475	1471	3.6	-	-
n-Tetradecanol	1674	1673	2.5	-	-
n-Hexadecanol	1878	1876	0.3	-	-

[a] RI calculated from retention times relative to those of n-alkanes (C_6-C_{32}) on the nonpolar HP-5 column.
[b] Percentages obtained by FID peak-area normalization.
[c] Identified by authentic samples.

was oxygenated monoterpene, at 43.5%. The numbers of the identified terpenoids were 22, 17, and 5 compounds in the percentages of 25.5%, 58.5%, and 8.4%, respectively. It could be concluded that the compositions of the volatile oils extracted from the flower, leaf, and stem had differences and that the leaf gave higher levels of terpenoids. However, the flower and stem parts of the oils were rich in nonterpenoid components, mostly aldehydes (39.1%, 79.8%), esters (11.4%, 5.1%), and hydrocarbons (5.1%, 2.5%), respectively. The results clearly indicate that the number of major constituents of the essential oil composition of the flower (n = 41) and leaf (n = 39) were very similar, but the stem oil was less volatile (n = 15).

In the literature, essential oil analysis of the root of *P. daurica* revealed 24 components,[14] compared to the 74 in our study, and 8 of them were the same compounds. Salicylaldehyde was the major constituent in both cases. Regarding the previously reported chemical composition of the roots of *Paeonia* species,[14–17] salicylaldehyde, *cis*-myrtanol, linalool, methyl salicylate, benzoic acid, and myrtenal were found to be major components with great compositional variation, as in the current study. The essential oils from *Paeonia* taxa of Turkish origin[14–15] seem quite similar to the Greek taxa[16] of *Paeonia*, since salicylaldehyde is the main component in most of their oils. The formulas of main components in the essential oils of *P. daurica* are shown in the Figure. It is necessary to point out that environmental factors, locations, and the parts of the plant strongly influence the chemical composition of the essential oil.

The antimicrobial activity of the essential oils of *P. daurica* was tested in vitro using the agar-well diffusion method[30–33] with the microorganisms listed in Table 3. The essential oils showed moderate antibacterial activity against 4 gram-positive (*S. aureus*, *E. faecalis*, *L. monocytogenes*, and *B. cereus*) and 3 gram-negative bacteria (*E. coli*, *Y. pseudotuberculosis*, and *P. aeruginosa*), a mycobacterium (*M. smegmatis*), and 2 pathogenic fungi (*C. albicans* and *S. cerevisiae*). The essential oil activity of the flower was more effective than that of the leaf or stem oil of *P. daurica* (Table 3), and was especially pronounced against gram-negative bacterium *E. coli*, gram-positive bacterium *S. aureus*, and the 2 fungi, *C. albicans* and *S. cerevisiae*.

Table 2. The chemical class distribution of the essential oil components of *P. daurica*.

Constituents	Flower		Leaf		Stem	
	% Area[a]	NC[b]	% Area[a]	NC[b]	% Area[a]	NC[b]
Terpenoids						
Monoterpene hydrocarbons	-	-	1.3	4	-	-
Oxygenated monoterpenes	0.6	1	43.5	5	8.4	5
Sesquiterpene hydrocarbons	11.4	10	1	2	-	-
Oxygenated sesquiterpenes	3.8	6	-	-	-	-
Diterpenoid	-	-	1	1	-	-
Terpene related compounds	9.7	5	11.7	5	-	-
Ester	11.4	4	7.6	6	5.1	1
Aldehyde	39.1	4	4.1	2	79.8	2
Hydrocarbons	5.1	6	7.2	11	2.5	5
Others	13.9	5	3.4	3	2.4	2
Total	95.0	41	80.8	39	98.2	15

[a] Percentages obtained by FID peak-area normalization.

[b] NC: Number of compounds.

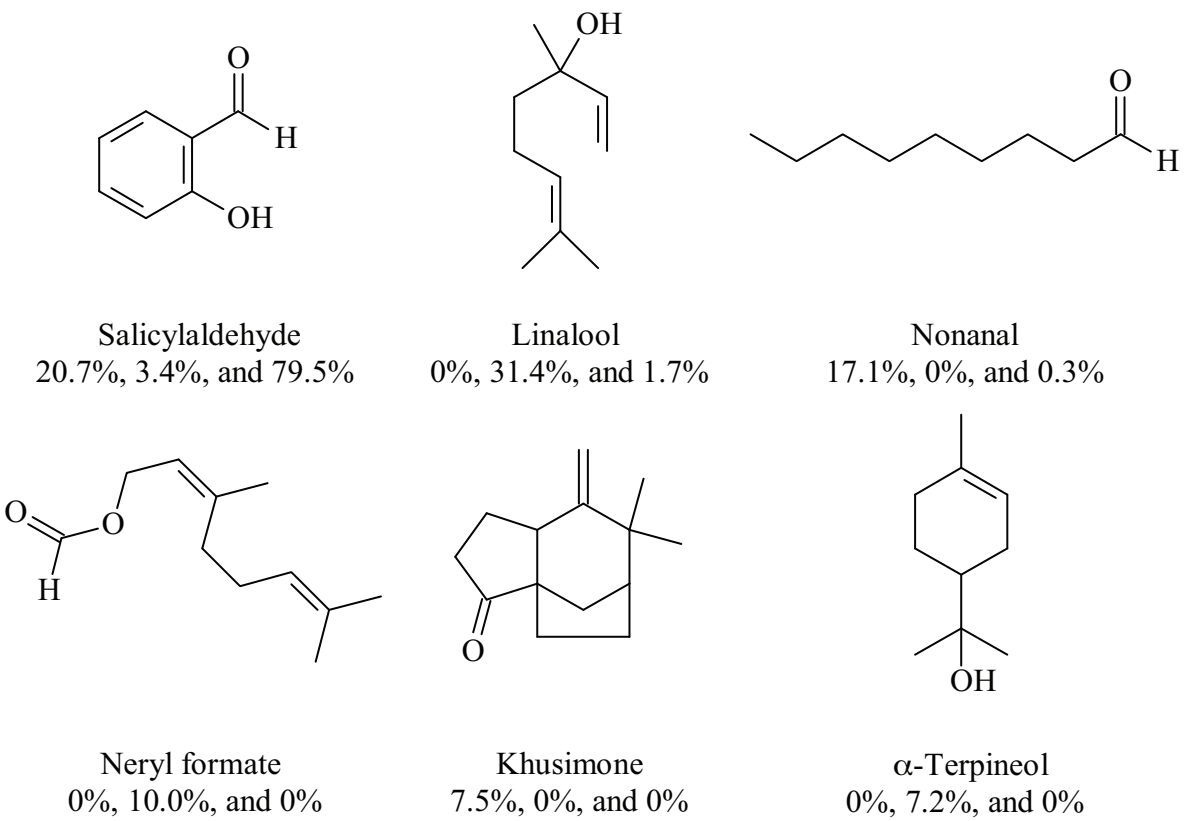

Figure. Main components in the essential oils from the flower, leaf, and stem of P. daurica, respectively.

Table 3. Screening results for antimicrobial activity of the essential oil from *P. daurica*.

Samples	Stock (μg/50 μL)	Microorganisms and inhibition zone (mm)									
		Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Li*	*Bc*	*Ms*	*Ca*	*Sc*
Flower	1000	12	11	10	25	8	10	10	10	20	30
Leaf	1000	8	7	-	8	-	-	8	12	6	10
Stem	1000	7	6	-	6	-	-	7	10	-	10
Amp.	10	10	18	18	35	10	10	15		-	-
Srp.	10								35		
Flu.	5									25	> 25

Ec: *Escherichia coli*, *Yp*: *Yersinia pseudotuberculosis*, *Pa*: *Pseudomonas aeruginosa*, *Sa*: *Staphylococcus aureus*, *Ef*: *Enterococcus faecalis*, *Li*: *Listeria monocytogenes*, *Bc*: *Bacillus cereus*, *Ms*: *Mycobacterium smegmatis*, *Ca*: *Candida albicans*, *Sc*: *Saccharomyces cerevisiae*, Amp.: Ampicillin, Srp.: Streptomycin, Flu.: Fluconazole, (-): no activity.

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