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RESEARCH ARTICLE

Essential oil compositions and antioxidant properties of the roots of twelve Anatolian *Paeonia* taxa with special reference to chromosome counts

Ilkay Orhan[1], Betül Demirci[2], Iman Omar[3], Huma Siddiqui[3], Erdal Kaya[4], M. Iqbal Choudhary[3], Gülay Ecevit-Genç[5], Neriman Özhatay[5], Bilge Şener[1], and K. Hüsnü Can Başer[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey, [2]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey, [3]H.E.J. Research Institute of Chemistry, ICCBS, University of Karachi, Pakistan, [4]Department of Ornamental Plant Breeding and Agronomy, Atatürk Central Horticultural Research Institute, Yalova, Turkey, and [5]Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, Turkey

Abstract

Essential oil compositions and antioxidant potentials of fourteen ethanol (75%) root extracts prepared from twelve taxa of the genus *Paeonia* (Paeoniaceae), including *P. arietina* Anders., *P. daurica* Andrews, *P. x kayae* N. Özhatay, *P. kesrouanensis* Thiéb., *P. mascula* (L.) Miller subsp. *arasicola* G. Kaynak, Ö. Yilmaz & R. Daşkin, *P. mascula* (L.) Miller subsp. *bodurii* N. Özhatay, *P.* cf. *mascula* L. (Mill.) subsp. *mascula* (two samples from central and northeastern Anatolia), *P.* cf. *officinalis* Retz., *P. peregrina* Miller (two samples from western and northwestern Anatolia), *P. tenuifolia* L., *P. turcica* Davis & Cullen, and *P. wittmanniana* Hartwiss ex Lindl. were assessed. The chromosome numbers of the root tips of the species were examined using chromosome staining technique with Shiff's reagent under Leitz microscope. The essential oils of the roots of the *Paeonia* species were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and the major components were identified as salicylaldehyde (10%–94.4%), *cis*-myrtanal (5.5%–59.7%), and methyl salicylate (2%–52.2%). Antioxidant potentials were tested against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide (NO) radicals using propyl gallate and rutin as the references. Total phenolic contents of the ethanol extracts were determined using Folin-Ciocalteau's method. The extracts exerted moderate NO scavenger effect and displayed insignificant DPPH radical scavenger activity at 500 μg mL^{-1}. On the other hand, *P. daurica*, *P. tenuifolia* and *P.* cf. *mascula* subsp. *mascula* are diploids with 2n = 10, while other nine taxa are tetraploids with 2n = 20.

Keywords: Paeonia*; essential oil; GC-MS; antioxidant; nitric oxide; DPPH; chromosome numbers*

Introduction

The genus *Paeonia* L. (Paeoniaceae), known as "şakayik, ayi gülü, bocur, etc." in Turkey, is only found in the northern hemisphere and comprises about 35 species. The genus has historically been subdivided into three sections, *Moutan* DC, *Onaepia* Lindley, and *Paeonia* DC (Davis & Cullen, 1965; Davis et al., 1988; Özhatay, 2000) and the section *Paeonia* is distributed in eastern and central Asia, the western Himalayas as well as the Mediterranean region. This section is composed of approximately 25 herbaceous species of both diploids and tetraploids (Hong et al., 2001), among them twelve taxa of *Paeonia* were recorded in Turkey, which is the most important gene center worldwide for this genus. Dependence on crucial income obtained by export of the plant means different species are thoughtlessly collected from the wild by digging out the roots

Address for Correspondence: I. Orhan, Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey. Tel: +90.312.2023186; Fax: +90.312.2235018. E-mail: iorhan@gazi.edu.tr

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in its natural habitat, which causes a slow extinction of the genus. Therefore, it has been decided to rigorously preserve the genus in Turkey by Atatürk Central Horticultural Research Institute in Yalova (Turkey) (personal communication).

Apart from its importance as an attractive ornamental plant, *Paeonia* (peony) species have also been utilized as medicinal plants. For instance, the Ottomans used *Paeonia* ssp. to treat internal diseases, pains, and epilepsy (Lew, 2002). Some of the peony species in Anatolia have been consumed as tea, which it has been recommended to drink two or three glasses per day against constipation and epilepsy as well as for antitussive purposes (Baytop, 1999). The genus *Paeonia* is also one of the most important crude drugs in Chinese traditional medicine used against atopic eczema as well as for anticoagulant, anti-inflammatory, analgesic, and sedative purposes (Kirby & Schmidt, 1997; Mutlugil, 1989).

We have previously performed some isolation and bioactivity studies on some *Paeonia* species (Yeşilada et al., 1992), and we also thought it worthwhile to ascertain essential oil composition and antioxidant potential of Turkish *Paeonia* species. We have herein examined the ethanol extracts prepared from the roots of *P. arietina, P. daurica daurica* (three samples from western and northeastern and southern Anatolia), *P.* x *kayae*, *P. kesrouanensis*, *P. mascula* subsp. *arasicola*, *P. mascula* subsp. *bodurii*, *P.* cf. *mascula* subsp. *mascula* (two samples from central and northeastern Anatolia), *P.* cf. *officinalis, P. peregrina* (two samples from western and northwestern Anatolia), *P. tenuifolia*, *P. turcica* and *P. wittmanniana* by 1,1-diphenylpicrylhydrazyl (DPPH) radical and nitric oxide (NO) scavenging activities at 500 μg mL^{-1} using an ELISA microplate reader. Total phenolic content (TPC) of the extracts was established by Folin-Ciocalteau's method.

Materials and methods

Plant materials

The roots of *P. arietina*, *P. daurica*, *P.x kayae*, *P. kesrouanensis*, *P. mascula* subsp. *arasicola*, *P. mascula* subsp. *bodurii*, *P.* cf. *mascula* subsp. *mascula* (two samples from central and northeastern Anatolia), *P.* cf. *officinalis, P. peregrina*, *P. tenuifolia*, *P. turcica*, and *P. wittmanniana* were obtained in 2007 at the experimental garden of Atatürk Central Horticultural Research Institute in Yalova (Turkey). All examined materials had been collected from the wild population and were planted in the experimental garden in Yalova. Population numbers of the species were given by Erdal Kaya, while the voucher specimens are housed in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE) (Table 1).

Table 1. Examined taxa with their voucher (ISTE) numbers, population numbers, locality and somatic chromosome numbers.

Species	Voucher number	Population number	Locality	2n
P. arietina	ISTE 84822	5801	Sivas; Zara	20
P. daurica	ISTE 84823	3301	Mersin; Tepeköy 1300 m	10
P. kesrouanensis	ISTE 84836	3101	Hatay; Yayladagi, 1100 m	20
P. masculas-ubsp. *bodurii*	ISTE 84824	1702	Çanakkale; Kalkim, 800-900 m	20
P. cf. *mascula* subsp. *mascula*	ISTE 84825	2901	Gümüşhane; Torul, 1750 m	10
P. cf. *mascula* subsp. *mascula*	ISTE 84826	4201	Konya; Doganhisar, 1650-1750 m	10
P. mascula subsp. *arasicola* (subsp. nova)	ISTE 84827	0302	Afyonkarahisar; Sultan mountain, 1400-1500 m	20
P.cf. officinalis	ISTE 84828	1708	Çanakkale; Kalkim	20
P. peregrina	ISTE 84829	1601	Bursa; M. Kemalpaşa, 500-600 m	-20
	ISTE 84830	1001	Balıkesir; Savaştepe	
P. peregrina	-	3901	Kırklareli; Dereköy, 350-400 m	- 20
	ISTE 84831	4501	Manisa; Spil mountain	
P. tenuifolia	ISTE 84832	2201	Edirne; Lalapaşa, Ortakça village	10
P. turcica	-	0701	Antalya; Elmali, 1800-1850 m	20
	ISTE 72288	-	Burdur; Altinyayla	
P. wittmanniana	ISTE 84837	5301	Rize; Ikizdere	20
P. x *kayae* (hybrid nova)	ISTE 84833	1703	Çanakkale; Yenice	20

Preparation of the extracts

The dried and powdered root materials of twelve above-mentioned *Paeonia* taxa were extracted with 300 mL (x 2) of ethanol (75%). All twelve pooled separately which were evaporated *in vacuo* until dryness to obtain the crude ethanol extracts which were later subjected to the antioxidant assays.

Isolation of the essential oils

Air-dried roots were crushed using a mortar and immediately hydrodistilled for 3 h using a Clevenger apparatus to provide essential oils. The oil yields are given in Table 2.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0. 25 mm,

Table 2. Composition of the essential oils of 14 Anatolian *Paeonia* samples.

RRI	Compound	*P. arietina* (%)	*P. daurica* (%)	*P. idea* (%)	*P. kesrounensias* (%)	*P. masculaΨ* (%)	*P. mascula* # (%)	*P. mascula* subsp. *arasicola* (%)	*P. mascula* subsp. *bodurii* (%)	*P. officinalis* (%)	*P. peregrina*** (%)	*P. peregrina** (%)	*P. tenuifolia* (%)	*P. turcica* (%)	*P. wittmanniana* (%)
1244	2-Pentyl furan	0.1	0.4	0.2	tr	tr	-	0.2	tr	tr	0.3	0.2	-	0.5	0.1
1541	Benzaldehyde	-	-	-	-	0.1	-	-	-	-	tr	0.1	-	tr	-
1547	Dihydroachillene	0.1	tr	tr	tr	-	tr	0.2	tr	0.2	tr	0.2	-	0.1	0.1
1548	(*E*)-2-Nonenal	0.1	0.2		tr	-	-	tr	tr	-	0.1	0.1	-	0.4	-
1560	*cis*-Myrtanal	16.7	9.7	15.4	3.3	1.7	10.3	27.9	7.4	42.2	45.7	16.6	59.7	5.5	7.2
1570	*trans*-Myrtanal	2.2	1.2	1.7	tr	0.2	1.2	3.2	0.9	5.1	6.6	2.2	6.9	0.8	0.9
1601	Nopinone	2.2	1.0	1.8	10.2	0.5	1.3	5.0	1.7	3.5	1.1	2.1	0.5	4.7	0.8
1614	Carvacrol methyl ether	tr	tr	tr	-	-	-	-	tr	-	-	0.1	-	tr	-
1641	Methyl benzoate	0.1	tr	-	-	-	tr	-	tr	-	-	0.1	-	0.1	-
1648	Myrtenal	4.2	1.2	2.5	2.7	0.1	0.7	3.9	2.4	3.4	3.5	2.1	1.7	1.7	0.5
1685	Ethyl benzoate	-	-	-	-	-	-	-	-		tr	0.1	-	-	-
1703	Salicylaldehyde	10.0	59.0	64.2	30.4	94.4	76.8	29.1	42.0	14.9	14.7	41.0	6.4	65.5	79.8
1729	α-Methyl cinnamaldehyde	2.3	1.8	3.2	3.4	tr	0.8	3.6	4.2	3.1	3.1	2.1	1.2	0.9	1.1
1744	Phellandral	0.5	tr	tr	-	tr	0.1	0.8	0.4	tr	0.5	0.4	0.3	0.3	0.2
1798	Methyl salicylate	52.2	10.1	8.3	-	2.0	1.7	15.9	20.5	17.8	3.3	16.1	10.6	1.5	1.0
1804	Myrtenol	0.2	-	-	-	tr	0.1	0.6	-	0.7	0.7	0.2	tr	0.3	0.3
1807	Perilla aldehyde	0.6	0.1	tr	1.8	tr	0.1	1.3	0.4	0.5	0.3	0.5	tr	0.6	0.3
1834	Ethyl salicylate	0.2	tr	-	tr	-	tr	-	tr	-	tr	0.1	-	-	-
1872	*cis*-Myrtanol	1.5	1.0	0.5	1.6	0.1	0.7	2.1	0.5	3.4	2.2	1.4	0.5	0.9	0.4
1879	*trans*-Myrtanol	0.7	0.5	0.8	1.3	0.1	0.3	4.2	1.0	3.7	1.7	1.8	0.4	1.0	0.2
1965	2-Ethyl hexanoic acid	0.1	-	-	-	-	tr	0.3	0.3	-		0.4	tr	0.1	-
2029	Perilla alcohol	0.3	0.3	-	1.3	tr	0.2	0.6	0.1	0.6	0.2	0.3	-	1.5	0.3
2186	Eugenol	-	-	-	-	tr	0.2	-	0.3	-	-	0.3	-	0.4	0.3
2198	Thymol	0.1	-	-	-	0.1	0.1	0.3	tr	0.2	-	0.9	-	tr	0.9
2239	Carvacrol	0.2	tr	1.1	-	0.1	0.4	0.3	1.4	-	tr	0.1	0.4	1.4	0.2

Table 2. Continued on next page.

Table 2. Continued.

RRI	Compound	*P. arietina* (%)	*P. daurica* (%)	*P. idea* (%)	*P. kesrounensias* (%)	*P. masculaΨ* (%)	*P. mascula#* (%)	*P. mascula* subsp. *arasicola* (%)	*P. mascula* subsp. *bodurii* (%)	*P. officinalis* (%)	*P. peregrina*** (%)	*P. peregrina** (%)	*P. tenuifolia* (%)	*P. turcica* (%)	*P. wittmanniana*
2242	Methyl hexadecanate	-	tr	-	tr	tr	0.1	-	0.4	-	tr	0.1	tr	0.2	tr
2250	Paenol	-	-	-	-	-	-	-	0.3	-	-	0.4	-	-	-
2262	Ethyl hexadecanate	-	-	-	-	tr	-	-	0.4	-	-	-	-	-	
2282	*cis*-Isoeugenol	tr	-	-	-	tr	0.1	tr	-	0.4	-	0.3	tr	0.2	1.4
2380	Hexyl cinnamic aldehyde	-	-	-	-	-	-		-	-	-	0.1	-	-	-
2384	1-Hexadecanol	0.1	-	-	-	tr	tr	tr	0.3	-	0.2	0.2	-	0.3	tr
2503	Dodecanoic acid	-	-	-	-	-	-	-	-	-		0.7	-	0.6	-
2509	Methyl linoleate	tr	0.7	-	1.6	tr	0.1	-	0.4	-	0.7	-	tr	0.2	tr
2538	Ethyl linoleate	-	-	-	-	tr	-	-	tr	-	-	-	-	-	-
2583	Methyl linolenate	-	-	-	tr		-	-	tr	-	-	-	-	0.1	-
2607	1-Octadecanol	-	-	-	-	tr	tr	-	-	-	-	-	-	0.3	-
2613	Ethyl linolenate	-	-	-	-	-	-	-	tr	-	-	-	-		-
2670	Tetradecanoic acid	-	tr	-	tr	-	tr	-	tr	-	-	0.8	-	tr	-
2804	Benzyl salicylate	tr	-	-	tr	-	tr	-	tr	-	-	0.3	-	0.7	0.5
2822	Pentadecanoic acid	tr	tr	-	tr	-	-	-	tr	-	-		0.9	0.9	-
2931	Hexadecanoic acid	4.0	11.4	-	34.1	0.3	4.1	-	13.1	-	14.9	6.3	8.4	5.6	3.3
	Total identified compound	28	24	15	21	26	28	21	34	17	24	34	19	34	24
	Total identified	98.7	98.6	99.7	91.7	99.7	99.4	99.5	98.4	99.7	99.8	98.7	97.9	97.3	99.8
	Oil yield (%)[‡]	trace	trace	trace	trace	0.22	0.03	0.01	trace	trace	trace	0.02	trace	trace	0.04

RRI: Relative retention indices calculated against *n*-alkanes; %, calculated from FID data; tr, (< 0.1%); *the northwestern Anatolian sample; **the western Anatolian sample; # the eastern Anatolian sample; Ψthe western Anatolian sample; [‡]essential oil yields are given on a moisture-free basis; trace, < 0.01%; -, not detected.

Table 3. Total phenolic contents (TPC) and percentage of DPPH and NO radical scavenging activity (%RSA) of the ethanolic root extracts from the *Paeonia* species.

Species	TPC (mg GA/100 mg extract)	%RSA of the extracts against DPPH*	%RSA of the extracts against NO*
P. arietina	6	48.34 ± 0.049	51.3 ± 0.003
P. daurica	10.3	31.45 ± 0.049	55.9 ± 0.354
P. idea	7.7	46.4 ± 0.006	55.6 ± 0.204
P. kesrouanensis	12.3	No activity	49.2 ± 0.009
P. mascula (The western Anatolian sample)	14.9	58.21 ± 0.006	56.9 ± 0.12
P. mascula (The eastern Anatolian sample)	5.3	46.11 ± 0.036	43.4 ± 0.28
P. mascula subsp. *arasicola*	14.1	29.81 ± 0.217	56.4 ± 0.047
P. mascula subsp. *bodurii*	14.3	No activity	56.3 ± 0.056
P. officinalis	7.9	53.03 ± 0.051	54.5 ± 0.003
P. peregrina (The western Anatolian sample)	4.2	44.36 ± 0.006	56.4 ± 0.038
P. peregrina (The northwestern Anatolian sample)	10.5	26.55 ± 0.026	54.4 ± 0.026
P. tenuifolia	5.4	39.05 ± 0.045	49.7 ± 0.109
P. turcica	7.6	23.69 ± 0.338	55.4 ± 0.017
P. wittmanniana	11	44.32 ± 0.055	56.3 ± 0.079
Standards			
Propyl gallate (DPPH)	-	90 ± 0.002	Not tested
Rutin (NO)	-	Not tested	79.9 ± 0.007

*n = 3 (Results of %RSA of the extracts and standards against DPPH and NO are expressed as average of three paralel experiments).

0.25 μm film thickness) was used with helium as carrier gas (0.8 mL min^{-1}). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

Gas chromatographic analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. In order to obtain the same elution order with GC-MS, simultaneous autoinjection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from the FID chromatograms.

Identification of components

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library; Adams Library; MassFinder 3 Library) (Jennings & Shibamoto, 1980; Adams, 2001; König et al., 2004) and in-house Başer Library of Essential Oil Constituents built up by genuine compounds and components of the known oils, as well as MS literature data (Joulain & König, 1988; McLafferty & Stauffer, 1989; ESO, 2000; Shaheen et al., 2005) were employed in identification of essential oil composition.

Antioxidant assays

Nitroprusside natrium was obtained from Merck 2549480 (Merck Co, Darmstadt, Germany), while sulfanilic acid from MP-Biomedicals (102990) (MP Biomedicals, CA, USA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and *N*-(1-naphthyl)ethylenediamine dihydrochloride and dimethyl sulfoxide (DMSO) were purchased from Sigma (St Louis, MO).

DPPH radical scavenging activity

The ethanol extracts were allowed to react with stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) for 1.5 h at 37°C as described in our previous publication (Badami et al., 2003). After incubation, decrease in absorption was measured at 515 nm using an ELISA multiplate reader (Spectra MAX-340). Percentage radical scavenging activity (RSA) by samples was determined in comparison with a DMSO-treated control group, and was calculated by using the following formula where "A" is referred to "Absorbance at 515 nm":

$$\%\,\mathrm{RSA} = 100 - \frac{(\mathrm{A}_{\text{test compound}})}{/\mathrm{A}_{\text{control}}} \times 100$$

NO scavenging activity

In the present experiment, modified Griess-Illosvoy reaction was employed by using *N*-(1-naphthyl)ethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%) (Skerget et al., 2005). The reaction mixture (200 μL) contained 10 μL of each sample, 20 μL of potassium phosphate buffer (0.1 mM, pH 7.4), and 70 μL of sodium nitroprusside (10 mM), then incubated at 25°C for 90 min. Following addition of 50 μL of *N*-(1-naphthyl) ethylenediamine dihydrochloride (0.1%, w/v) into the reaction mixture given above, 50 μL of sulfanilic acid reagent was then added and shaken carefully. Absorbance at 540 nm against the blank solution containing all reagents except for the test sample was determined in a microtiter

plate by ELISA reader. RSA was calculated by using the above formula for DPPH assay.

Total phenolic contents (TPC) in the extracts

The concentration of total phenols in the extracts was determined by UV spectrophotometry using Folin-Ciocalteau's reagent (Singleton & Rossi, 1965). The absorbance was measured at 760 nm and the results obtained were expressed in mg of gallic acid (GA) per 100 mg of each extract (mg GA/100 mg extract).

Karyological study

All cytological observations were made from the root tips of the materials shown in Table 1. The seeds were germinated on soil in pots. Fresh root tips were cut about 1 cm long, pretreated in 1-bromonaphthalene at 4°C for 24 h, then fixed with 1:3 (glacial acetic acid:absolute alcohol) at 4°C for 24 h. The root tips were hydrolyzed in 1 N HCl at 60°C for 10 min and stained in Shiff's reagent. Stained root tips were squashed with a drop of 2% aceto orcein. Permanent slides were made by mounting with Sandeural. Preparations were examined using a Leitz microscope.

Results and discussion

Antioxidant potentials of the ethanol extracts of the root samples of *P. arietina, P. daurica P. x kayae*, *P. kesrouanensis*, *P. mascula* subsp. *arasicola*, *P. mascula* subsp. *bodurii*, *P.* cf. *mascula* subsp. *mascula* (two samples from central and northeastern Anatolia), *P.* cf. *officinalis, P. peregrina* (two samples from the western and northwestern Anatolia), *P. tenuifolia*, *P. turcica*, and *P. wittmanniana* were determined by their radical scavenger activities against DPPH and NO along with their total phenolic contents (Table 3).

Our results demonstrate the *Paeonia* species screened here showed a moderate anti-radical activity against DPPH causing inhibition between 23.9% and 58.2% as compared to the propyl gallate (90%), and they displayed similar activity against NO ranging between 43.4% and 56.9% (Table 2). TPCs were the highest in *P.* cf. *mascula* subsp. *mascula* of the central Anatolia (14.9 mg), followed by *P. mascula* subsp. *bodurii* (14.3 mg), and *P. mascula* subsp. *arasicola* (14.1 mg). Interestingly, *P.* cf. *mascula* subsp. *mascula* of the northeastern Anatolia was found to contain approximately one third the amount (5.3 mg) of the central sample, which may depend on various factors such as climate conditions, soil composition, etc. The same difference was also observed between two *P. peregrina* samples collected from the western and northwestern regions of Turkey, which may well be due to the above-mentioned factors. The sample of *P.* cf. *mascula* subsp. *mascula* obtained from central Anatolia that has the highest TPC among the others also exerted the highest antioxidant capacity against DPPH and NO radicals.

The results obtained by essential oil analyses have highlighted great compositional variation in all fourteen oils in which salicylaldehyde, *cis*-myrtanal, and methyl saliciylate were the major components (Table 2). Salicylaldehyde was dominant in *P.* cf. *mascula* subsp. *mascula* samples of central and northeastern Anatolia, *P. x kayae*, *P. daurica*, *P. mascula* subsp. *bodurii*, *P. peregrina* of northwestern Anatolia, *P. turcica*, and *P. wittmanniana*, whereas methylsalicylate was the leading component only in *P. arietina*. *Cis*-myrtanal was the major compound in *P. tenuifolia* (59.7%), followed by *P. peregrina* of west Anatolia (45.7%), and *P.* cf. *officinalis* (42.2%).

Chromosome numbers are known to be important to improve agriculturally important plants and to determine relationships between plants. Since *Paeonia* species are also considered one of the ornamentally important plants and no karyological study has been so far performed on the genus, we determined their chromosome numbers. Our karyological results indicated that the somatic chromosome numbers of the species of *Paeonia* is either 2n = 10 or 2n = 20. *P. daurica P. tenuifolia* and *P.* cf. *mascula* subsp. *mascula* are diploids with 2n = 2x = 10. The other nine taxa are tetraploids with 2n = 4x = 20 (Table 1). Considering their essential oil compositions, there has been no correlation observed between diploid and tetraploid taxa.

There have been several reports on the antioxidative effect of *Paeonia* species of Chinese origin. For instance; in a study (Kirby & Schmidt, 1997), an herbal mixture used as a tea in China consisting of many Chinese plants including *P. lactiflora*, was tested for each ingredient plant separately against DPPH radical and superoxide (SO) anion for their antioxidant effects as well as the tea itself. Among them, the aqueous extract of *P. lactiflora* was found to be the most active against DPPH, whereas it was one of the inactive plants towards SO. Conversely, seventy Chinese medicinal plants were screened for their SO and hydroxyl radical scavenging activities and *P. lactiflora* potently scavenged SO radical (Liu & Ng, 2000). In another study, twelve Chinese medicinal plants including *P. suffruticosa* were screened for their antioxidant activity through lipid peroxidation in rat kidney and brain homogenates, SO and hydroxyl radical scavenging effects (Lee et al., 2003) and *P. suffruticosa* strongly inhibited generation of hydroxyl and SO radicals at 100 $\mu g\ mL^{-1}$. It was also one of the most potent plants in lipid peroxidation, where it had a very low pro-oxidant effect. *P. suffruticosa* was reported to have a very high level of DPPH radical scavenging activity causing over 90% of inhibition (Li et al., 2008). Also, this plant induced

43%, 28%, and 45% increases in superoxide dismutase, catalase, and gluthation peroxidase levels, respectively. On the other hand, *P. lactiflora* and *P. suffruticosa* were found to contain 26.75 and 24.51 mg TPC expressed as GA equivalent/g extract, and these two plants were concluded to possess high antioxidant capacity in scavenging free radicals and reducing (Miyazawa et al., 1983).

However, there have been few reports on volatile constituents of *Paeonia* species. Although it is a well-reputed Chinese traditional plant, only two papers mention the volatile oils of *P. lactiflora* and *P. suffruticosa* of Chinese origin, in which benzoic acid was the dominant component (Miyazawa et al., 1984; Papandreou et al., 2002). The essential oils of three taxa including *P. clusii* subsp. *clusii*, *P. mascula* subsp. *hellenica*, and *P. parnassica* growing in Greece were analyzed by GC-MS (Ivanova et al., 2002), and among the twelve components identified, salicylaldehyde was found to be the major one all in three species. Methyl salicylate was the second major constituent in *P. parnassica* (24.7%), while the second one was paeonol in *P. clusii* subsp. *clusii* (32.6%). Benzoic acid, the major compound found in Chinese *P. lactiflora* and *P. suffruticosa*, was only found in *P. clusii* subsp. *clusii* constituting 7.7% of the oil. Interestingly, salicylaldehyde, the main compound in the essential oils of the Greek and some of the Turkish *Paeonia* species mentioned herein, has been never reported from Chinese peony roots. Also, benzoic acid and its several derivatives were observed to dominate the essential oils from *P. peregrina* and *P. tenuifolia* growing in Bulgaria, which are consistent with their Chinese counterparts (Ivanova et al., 2002). However, benzoic acid was not detected at all in the essential oils of our *Paeonia* species.

Considering the literature on *Paeonia*, except for two Chinese *Paeonia* species (*P. lactiflora* and *P. suffruticosa*), there has been so far no information about the antioxidant potential of other members of the genus *Paeonia* of non-Chinese origin. Accordingly, the essential oils from *Paeonia* taxa of Turkish origin seem quite similar to the Greek taxa of *Paeonia* since salicylaldehyde is the main component in most of their oils. To the best of our knowledge, this is the first report on antioxidant properties of these twelve *Paeonia* taxa and their total phenolic contents along with the chromosome numbers and essential oil compositions of the Turkish *Paeonia* taxa.

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References

Adams RP (2001): *Identification of Essential Oil Components by Gas Chromatography-Quadrupole Mass Spectroscopy*. Allured Publishing, IL, USA.

Badami S, Gupta MK, Suresh B (2003): Antioxidant activity of the ethanolic extract of *Striga orobanchioides*. *J Ethnopharmacol* 85: 227–230.

Baytop T (1999): *Therapy with Plants in Turkey (Past and Present)*. Istanbul, Nobel Medical Bookhouse, pp. 387–388.

Davis PH, Cullen J (1965): *Paeonia* L, in: Davis PH, ed., *Flora of Turkey and the East Islands*, Vol. I. Edinburgh, Edinburgh University Press, pp. 204–206.

Davis PH, Mill RR, Tan K (1988): *Paeonia* L., in: Davis PH, ed., *Flora of Turkey and the East Islands*, Vol. X. Edinburgh, Edinburgh University Press, pp. 22–23.

ESO (2000): *The Complete Database of Essential Oils*. The Netherlands, Boelens Aroma Chemical Information Service.

Hong DY, Pan KY, Turland NJ (2001): *Paeoniaceae*, in: Wu ZY & Raven PH, eds, *Flora of China*, Vol. VI. Science Press and Missouri Botanic Garden Press, St. Louis, USA. pp. 127–132.

Ivanova A, Delcheva I, Tsvetkova I, Kujumgiev A, Kostova I (2002): GC-MS analysis and antimicrobial activity of acidic fractions obtained from *Paeonia peregrina* and *Paeonia tenuifolia* roots. *Z Naturforsch* 57c: 624–628.

Jennings WG, Shibamoto T (1980): Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC. New York, Academic Press.

Joulain D, König WA (1988): The Atlas of Spectra Data of Sesquiterpene Hydrocarbons. Hamburg, E.B. Verlag.

Kirby AJ, Schmidt RJ (1997): The antioxidant activity of Chinese herbs for eczema and of placebo herbs – I. *J Ethnopharmacol* 56: 103–108.

König D, Joulain D, Hochmuth DH (2004): Terpenoids and Related Constituents of Essential Oils. MassFinder 3. Hochmuth DH (Ed.). Convenient and Rapid Analysis of GC-MS, incorporating W.A. Hamburg, Germany.

Lee SE, Hwang H, Ha JS, Jeong HS, Kim JH (2003): Screening of medicinal plant extracts for antioxidant activity. *Life Sci* 73: 167–179.

Lew E (2002): Reconstructed Materia Medica of the medieval and Ottoman Al-Sham. *J Ethnopharmacol* 30: 167–179.

Li HB, Wong CC, Cheng KW, Chen F. (2008): Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. *LWT-Food Sci Technol 41:385–390*.

Liu F, Ng TB (2000): Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci* 66: 725–735.

McLafferty FW, Stauffer DB (1989): The Wiley/NBS Registry of Mass Spectral Data. New York, Wiley.

Miyazawa M, Maruyama H, Kameoka H (1983): Essential oil constituents of *Moutan radicis cortex* (*Paeonia moutan* SIMS.) (= *P. suffruticosa* Andrews). *Agric Biol Chem* 47: 2925–2927.

Miyazawa M, Maruyama H, Kameoka H (1984): Essential oil constituents of *Paeonia radix* (*Paeonia lactiflora* Pall.) (*Paeonia albiflora* Pall.). *Agric Biol Chem* 48: 2847–2849.

Mutlugil A (1989): Pharmacognosic researches on the underground parts of *Paeonia daurica* Andrews. PhD thesis for the Institute of Health Sciences of Gazi University, Ankara, Turkey.

Özhatay N (2000): *Paeonia* L., in: Güner A., Özhatay N, Ekim T, Başer KHC, eds, *Flora of Turkey and the East Islands*, Vol. XI (Suppl.). Edinburgh, Edinburgh University Press, pp. 15–16.

Papandreou V, Magiatis P, Chinou I, Kalpoutzakis E, Skaltsounis AL, Tsarbopoulos A (2002): Volatiles with antimicrobial activity from the roots of Greek *Paeonia* taxa. *J Ethnopharmacol* 81: 101–104.

Shaheen F, Ahmad M, Khan MTH, Jalil S, Ejaz A, Sultankhodjaev MN, Arfan M, Choudhary MI, Atta-ur-Rahman (2005): Alkaloids of *Aconitum laeve* Royle and their anti-inflammatory, antioxidant and tyrosinase inhibition activities. *Phytochemistry* 66: 935–940.

Singleton VL, Rossi JA Jr (1965): Colorimetry of total phenolics with phosphomolibdic-phosphotungtic acid reagents. *Am J Enol Viticult* 16: 144–158.

Skerget M, Kotnik P, Hadolin M, Hras AR, Simonic M, Knez Z (2005): Phenols, proanthocyanidins, flavones, and flavonols in some plant materials and their antioxidant activities. *Food Chem* 89: 191–198.

Yeşilada E, Mutlugil A, Şener B (1992): The anti-inflammatory principle of the roots of *Paeonia daurica* Andrews. *Int J Pharmacog* 30: 66–70.