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Composition of the essential oils of Pinus nigra Arnold from Turkey

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Essential oil composition of the needles of *Pinus nigra* Arnold collected from different localities in Turkey was investigated by GC and GC/MS. The main components in the oils were α-pinene, β-pinene, β-caryophyllene, and germacrene D.

Key Words: *Pinus nigra*, Pinaceae, essential oil, α-pinene, β-pinene, β-caryophyllene, germacrene D, chemotaxonomy.

Introduction

The genus *Pinus* (Pinaceae) is represented in Turkey by 5 species: *Pinus nigra, P. brutia, P. sylvestris, P. halepensis,* and *P. pinea. P. nigra* is distributed in Anatolia, Cyprus, Crimea, West Caucasus, Balkans, the South Carpathians, and Western Syria.[1]

P. nigra is a tree, up to 30 m tall. Bark dark gray to blackish. Young shoots glabrous. Leaves 70-180 × 2 mm, dark green, rigid, often curved. Buds resinous. Cone ovoid, erect or ascending. Scales flattened, apophysis ± flat. Umbo mucronate. Frequently a dominant tree, or in scrub, from 300-1800 m, in the south not usually below 1000 m.[1]

Conifers are a renewable source of essential oils. Components of the essential oils, besides their economic value, seem to play an important role in the plant defense system against fungus and insect attacks.

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Some studies have been carried out to observe the effect on the seasonal, genotypic, and environmental variability of the chemical contents in *Pinus* species.[2−28] The effects of geographical variations in the needle oil composition of *P. nigra* have also been reported.[29−31] Chemical compositions of the essential oils of the needles of *P. nigra* have been published.[32−35]

In the present study, we report on variations in the yield and compositions of the needle oils of *P. nigra* collected at different periods from various sites in Turkey.

Experimental

Plant Material and Hydrodistillation. Young needles of P. nigra Arn. were collected from different regions in different seasons (Table 1). Each sample was hydrodistilled using a Clevenger type apparatus. The oil was collected for 3 h after the first drop of the distillate eluted from a cold finger. The oils were then kept refrigerated until the GC and GC-MS analyses.

Table 1. The collecting sites and dates.

BURSA	KÜTAHYA	DENİZLİ	ANTALYA	İÇEL	SİNOP	KASTAMONU	SAMSUN
20.09.1994	21.09.1994	08.06.1994	09.06.1994	16.06.1994	04.08.1994	08.08.1994	25.05.1994
25.12.1994	27.12.1994	10.09.1994	16.09.1994	14.09.1994	07.11.1994	11.11.1994	18.08.1994
31.03.1995	02.04.1995	16.12.1994	24.12.1994	05.12.1994	20.02.1995	05.02.1995	17.11.1994
27.07.1995	28.07.1995	01.03.1995	09.03.1995	15.02.1995	25.05.1995	29.05.1995	17.02.1995

GC and GC-MS Analysis. The oils were analyzed by GC and GC-MS using a Hewlett-Packard GCD system. A Thermon 600T column (50 m $\times$ 0.25 mm ϕ with 0.25 μm film thickness) was used, with nitrogen as carrier gas. GC oven temperature was kept at 70 °C for 10 min and programmed to 180 °C at rate of 2 °C/min and then kept constant at 180 °C for 30 min. The split ratio was adjusted at 60:1. The injector and detector temperatures were 250 °C. MS were taken at 70eV. Mass range was from m/z 10 to 400. A library search was carried out using the NBS/NIH/EPA Library and the BASER Library of Essential Oil Constituents. Relative percentage amounts were calculated from the FID results. Oil yields are given on moisture-free basis (Table 2).

Results and discussion

The 42 compounds identified in the oils are listed in Tables 3-10.

The highest oil yields were obtained in the materials collected during summer (June to August) (between 0.38% and 1.03%).

The amounts of α-pinene in the oils were always higher than those of β-pinene. The total contents of α-pinene and β-pinene ranged between 52% and 67% in the samples collected from the south (Antalya, İçel), between 45% and 57% in samples from the west (Bursa, Denizli) and between 30% and 43% in samples from the north (Sinop, Kastamonu, Samsun). The contents of major 4 constituents in the oils are given in Table 11.

Table 2. Oil yields of *P. nigra* samples.

BURSA		KÜTAHYA		DENİZLİ		ANTALYA	
Date	%	Date	%	Date	%	Date	%
20.09.1994	0.43	21.09.1994	0.43	08.06.1994	0.44	09.06.1994	0.55
25.12.1994	0.49	27.12.1994	0.55	10.09.1994	0.33	16.09.1994	0.49
31.03.1995	0.33	02.04.1995	0.70	16.12.1994	0.22	24.12.1994	0.38
27.07.1995	0.65	28.07.1995	1.03	01.03.1995	0.06	09.03.1995	0.49

İÇEL		SİNOP		KASTAMONU		SAMSUN	
Date	%	Date	%	Date	%	Date	%
16.06.1994	0.65	04.08.1994	0.51	08.08.1994	0.67	25.05.1994	0.28
14.09.1994	0.43	07.11.1994	0.16	11.11.1994	0.54	18.08.1994	0.38
05.12.1994	0.38	20.02.1995	0.38	05.02.1995	0.44	17.11.1994	0.22
15.02.1995	0.54	25.05.1995	0.65	29.05.1995	0.54	17.02.1995	0.16

Table 3. Results of analysis of *P. nigra* needle oils from İçel.

Collection Dates Compounds	*14.9.1994* %	*5.12.1994* %	*15.2.1995* %	*16.6.1994* %
tricyclene	0.04	0.12	0.13	0.05
$\alpha-$pinene	22.20	43.05	34.18	23.36
camphene	0.41	0.66	0.87	0.34
hexenal	0.01	0.04	0.03	0.12
$\beta-$pinene	24.80	23.34	22.40	34.10
sabinene	0.07	0.08	0.11	0.04
Δ^3-carene	—	0.09	—	—
myrcene + α-phellandrene	1.33	0.93	1.19	1.92
$\alpha-$terpinene	0.01	0.02	0.01	0.03
limonene	4.01	1.18	1.53	7.10
$\beta-$phellandrene	1.13	0.85	1.21	1.05
2-pentylfuran	0.03	trace	0.03	0.04
(Z)- β-ocimene	0.02	0.01	0.02	0.02
$\gamma-$terpinene	0.05	0.03	0.03	0.05
(E)- β-ocimene	0.53	0.43	0.80	0.21
P-cymene	0.06	0.03	0.10	0.04
terpinolene	0.29	0.25	0.19	0.27
campholene aldehyde	0.07	0.05	0.02	0.03
$\alpha-$copaene	1.16	0.12	0.07	0.08
linalool	0.14	0.06	0.06	0.14

Table 3. Contunied.

Collection Dates Compounds	*14.9.1994* %	*5.12.1994* %	*15.2.1995* %	*16.6.1994* %
linalyl acetate	0.32	0.07	0.19	0.21
longifolene	0.39	0.07	0.49	0.54
bornyl acetate	0.22	0.21	0.26	0.07
$\beta-$elemene	0.04	0.02	0.09	0.05
$\beta-$caryophyllene	9.21	6.13	5.65	6.51
hexadecane	0.09	0.05	0.17	0.08
myrtenal	0.48	0.08	0.26	0.33
trans-pinocarveol	0.69	0.15	0.52	0.41
$\alpha-$humulene	1.54	0.94	0.95	1.11
$\alpha-$terpineol	0.48	0.17	0.39	0.26
$\alpha-$terpinyl acetate	1.52	0.40	3.17	1.32
germacrene-D	14.48	14.91	12.79	6.45
$\alpha-$muurolene	0.65	0.25	0.46	0.31
$\delta-$cadinene	2.40	1.77	0.93	1.13
dodecanol	0.99	0.20	0.46	0.75
Caryophyllene oxide	trace	0.02	0.13	trace
Methyl eugenol	0.05	0.02	Trace	—
trans-methyl isoeugenol	0.14	0.06	0.10	0.08
T-muurolol	0.34	0.14	0.23	0.19
farnesyl acetate	0.21	0.07	0.12	0.03
TOTAL	92.00	98.06	90.87	89.42

Trace: <0.01%

Table 4. Results of analysis of *P. nigra* needle oils from Antalya.

Collection Dates Compounds	9.6.1994 %	*16.9.1994* %	*24.12.1994* %	9.3.1995 %
tricyclene	0.11	0.13	0.09	0.12
$\alpha-$pinene	34.38	37.03	24.40	44.16
camphene	0.72	0.88	0.43	0.94
hexenal	0.07	0.06	0.10	0.04
$\beta-$pinene	17.10	17.22	21.89	14.05
sabinene	0.08	0.11	0.06	0.10
Δ^3-carene	0.01	0.04	0.03	0.01
Myrcene + α-phellandrene	0.95	1.07	1.58	0.95
$\alpha-$terpinene	0.02	0.03	0.01	0.02

Table 4. Contunied.

Collection Dates Compounds	9.6.1994 %	*16.9.1994* %	*24.12.1994* %	9.3.1995 %
limonene	1.01	1.40	0.84	1.26
$\beta-$phellandrene	0.97	0.74	0.82	0.66
2-pentylfuran	0.04	0.03	0.04	0.02
(Z)- β-ocimene	0.03	0.04	0.01	0.01
$\gamma-$terpinene	0.04	0.05	0.03	0.04
(E)- β-ocimene	0.65	0.67	0.64	0.34
P-cymene	0.05	0.06	0.04	0.05
terpinolene	0.20	0.25	0.09	0.17
campholene aldehyde	0.08	0.07	0.07	0.06
$\alpha-$copaene	0.17	0.15	0.15	0.14
linalool	0.43	0.57	0.03	0.06
linalyl acetate	0.07	0.14	0.22	0.16
longifolene	1.85	2.72	0.05	0.24
bornyl acetate	0.58	0.83	0.30	0.25
$\beta-$elemene	trace	0.06	0.03	0.05
$\beta-$caryophyllene	7.84	6.40	8.42	7.36
hexadecane	0.06	0.11	Trace	trace
myrtenal	0.11	0.07	0.31	0.17
trans-pinocarveol	0.20	0.65	0.74	0.36
$\alpha-$humulene	1.30	1.11	1.43	1.15
$\alpha-$terpineol	0.31	0.40	0.52	0.76
$\alpha-$terpinyl acetate	2.13	2.75	0.51	1.14
germacrene-D	13.67	7.97	18.03	10.37
$\alpha-$muurolene	0.61	0.55	0.53	0.47
$\delta-$cadinene	3.70	1.93	2.04	2.05
dodecanol	0.54	0.60	0.59	0.95
Caryophyllene oxide	0.09	0.07	0.03	0.02
Methyl eugenol	0.07	0.11	0.17	0.05
trans-methyl isoeugenol	0.11	0.11	0.12	0.17
T-muurolol	0.32	0.26	0.08	0.29
farnesyl acetate	0.20	0.08	0.05	0.07
TOTAL	92.49	88.68	86.96	90.38

Trace: <0.01%

Table 5. Results of analysis of *P. nigra* needle oils from Denizli.

Collection Dates	8.6.1994	10.9.1994	16.12.1994	1.3.1995
Compounds	%	%	%	%
tricyclene	0.08	0.18	0.13	0.03
$\alpha-$pinene	32.12	49.63	38.83	4.51
camphene	0.56	0.93	0.90	0.25
hexenal	0.06	0.04	0.06	trace
$\beta-$pinene	13.07	2.12	10.06	1.42
sabinene	0.17	0.13	0.22	0.05
Δ^3-carene	0.02	0.01	0.03	—
myrcene+ α-phellandrene	0.73	0.84	0.69	0.12
$\alpha-$terpinene	0.03	0.02	0.03	0.06
limonene	0.92	1.19	1.01	0.35
$\beta-$phellandrene	0.61	0.38	0.57	0.26
2-pentylfuran	0.06	0.02	0.04	0.08
(Z)- β-ocimene	0.02	0.02	0.01	—
$\gamma-$terpinene	0.04	0.03	0.05	—
(E)- β-ocimene	0.36	0.61	0.42	0.08
P-cymene	0.08	0.05	0.09	0.06
terpinolene	0.19	0.08	0.16	0.12
campholene aldehyde	0.20	0.15	0.14	0.52
$\alpha-$copaene	0.39	0.30	0.29	0.89
linalool	0.20	0.17	0.04	trace
linalyl acetate	0.18	0.08	0.24	0.10
longifolene	0.68	0.06	0.06	0.28
bornyl acetate	0.19	0.24	0.25	trace
$\beta-$elemene	0.04	0.01	0.06	0.16
$\beta-$caryophyllene	7.42	5.87	9.44	21.48
hexadecane	0.13	trace	0.13	0.19
myrtenal	0.30	0.11	0.24	0.14
trans-pinocarveol	0.47	0.20	0.49	0.28
$\alpha-$humulene	1.35	1.08	1.71	3.64
$\alpha-$terpineole	3.35	2.69	0.55	0.54
germacrene isomer	3.35	2.69	2.33	8.18
$\alpha-$terpinyl acetate	1.66	0.76	0.38	2.56
germacrene- D	11.69	18.43	14.01	11.23
$\alpha-$muurolene	1.00	0.80	1.01	1.97
$\delta-$cadinene	7.15	4.65	4.28	16.81
dodecanol	0.77	0.40	0.97	2.47
Caryophyllene oxide	0.07	0.05	0.06	0.27
Methyl eugenol	2.01	0.02	0.04	0.71
trans-methylisoeugenol	0.23	0.15	0.15	0.67
T-muurolol	0.45	0.35	0.31	1.22
farnesyl acetate	0.17	0.11	0.09	0.24
TOTAL	92.57	95.65	90.57	81.97

Trace: <0.01%

Table 6. Results of analysis of *P. nigra* needle oils from Kütahya.

Collection Dates	21.9.1994	27.12.1994	2.4.1995	28.7.1995
Compounds	%	%	%	%
tricyclene	0.09	0.12	0.11	0.10
$\alpha-$pinene	24.69	31.93	45.09	44.39
camphene	0.47	0.56	0.77	0.73
hexenal	0.05	0.06	0.05	0.04
$\beta-$pinene	17.63	14.34	10.22	14.48
sabinene	0.07	0.12	0.10	0.11
Δ^3-carene	—	0.01	—	—
myrcene + α-phellandrene	0.76	0.93	1.24	1.24
$\alpha-$terpinene	0.01	0.03	0.02	0.04
limonene	0.59	0.74	1.19	1.11
$\beta-$phellandrene	0.64	0.58	0.94	0.95
2-pentylfuran	0.02	0.02	0.02	0.01
(Z)- β-ocimene	0.02	0.02	0.01	0.03
$\gamma-$terpinene	0.03	0.04	0.05	0.06
(E)- β-ocimene	0.27	0.38	0.70	1.31
P-cymene	0.04	0.06	0.07	0.03
terpinolene	0.08	0.14	0.15	0.40
campholene aldehyde	0.25	0.26	0.11	0.08
$\alpha-$copaene	0.44	0.46	0.24	0.18
linalool	0.31	0.21	0.02	0.09
linalyl acetate	0.23	0.16	0.07	0.03
longifolene	1.32	0.91	0.05	0.41
bornyl acetate	0.15	0.20	0.31	0.29
$\beta-$elemene	0.02	0.01	0.03	0.01
$\beta-$caryophyllene	5.26	6.12	10.19	7.90
hexadecane	0.07	0.07	0.06	0.06
myrtenal	0.45	0.30	0.11	0.04
trans-pinocarveol	0.52	0.38	0.40	0.08
$\alpha-$humulene	1.35	1.41	1.81	1.35
$\alpha-$terpineole	0.58	0.44	0.26	0.23
germacrene isomer	3.96	3.97	2.04	1.43
$\alpha-$terpinyl acetate	3.82	2.76	0.44	2.48
germacrene- D	15.43	12.24	12.97	11.27
$\alpha-$muurolene	0.96	1.02	0.68	0.40
$\delta-$cadinene	7.82	7.69	3.48	2.48
Dodecanol	0.58	0.60	0.39	0.31
Caryophyllene oxide	trace	trace	Trace	0.02
Methyl eugenol	0.04	0.04	0.02	0.06
trans-methyl isoeugenol	0.21	0.26	0.10	0.09
T-muurolol	0.43	0.45	0.22	0.19
farnesyl acetate	0.12	0.15	0.09	0.09
TOTAL	89.78	90.19	94.83	94.60

Trace: <0.01%

Table 7. Results of analysis of *P. nigra* needle oils from Bursa.

Collection Dates	20.9.1994	25.12.1994	31.3.1995	27.7.1995
Compounds	%	%	%	%
tricyclene	0.21	0.19	0.11	0.17
$\alpha-$pinene	40.87	39.21	38.13	35.94
camphene	0.90	0.86	0.76	0.84
hexenal	0.04	0.03	0.04	0.06
$\beta-$pinene	3.11	5.85	12.16	19.52
sabinene	0.11	0.17	0.11	0.04
Δ^3-carene	—	—	0.01	0.04
myrcene + α-phellandrene	0.74	0.72	0.73	1.03
$\alpha-$terpinene	0.01	0.01	0.03	0.09
limonene	0.80	0.83	0.73	1.02
$\beta-$phellandrene	0.52	0.58	0.60	0.81
2-pentylfuran	0.02	0.02	0.02	—
(Z)- β-ocimene	0.02	0.02	0.01	0.03
$\gamma-$terpinene	0.03	0.03	0.05	0.11
(E)- β-ocimene	0.42	0.37	0.63	1.56
P-cymene	0.05	0.06	0.05	0.05
terpinolene	0.08	0.11	0.22	0.70
campholene aldehide	0.11	0.12	0.14	0.13
$\alpha-$copaene	0.25	0.26	0.27	0.26
linalool	0.04	0.16	0.05	0.03
linalyl acetate	0.09	0.01	0.09	0.06
longifolene	0.15	0.04	0.17	0.09
bornyl acetate	0.22	0.27	0.45	0.52
$\beta-$elemene	—	0.02	0.02	0.03
$\beta-$caryophyllene	8.36	8.76	7.80	6.34
hexadecane	0.33	0.04	0.08	0.12
myrtenal	0.16	0.22	0.11	0.09
trans-pinocarveol	0.29	0.44	0.24	0.16
$\alpha-$humulene	1.46	1.59	1.39	1.08
$\alpha-$terpineol	0.73	0.94	0.36	0.24
germacrene isomer	2.33	2.49	2.48	2.16
$\alpha-$terpinyl acetate	1.50	1.41	1.25	1.06
germacrene- D	21.38	18.41	15.80	13.07
$\alpha-$muurolene	0.71	0.93	0.83	0.67
$\delta-$cadinene	3.93	4.28	4.81	4.36
dodecanol	0.67	0.72	0.51	0.32
Caryophyllene oxide	trace	trace	Trace	trace
Methyl eugenol	0.05	trace	0.05	0.04
trans-methyl isoeugenol	0.20	0.19	0.16	0.12
T-muurolol	0.39	0.42	0.39	0.27
farnesyl acetate	0.15	0.19	0.10	0.08
TOTAL	91.42	90.86	90.68	93.31

Trace: <0.01%

Table 8. Results of analysis of *P. nigra* needle oils from Kastamonu.

Collection Dates	5.2.1995	8.8.1994	11.11.1994	29.5.1995
Compounds	%	%	%	%
tricyclene	0.20	0.18	0.12	0.08
$\alpha-$pinene	29.31	33.12	30.31	35.53
camphene	1.08	0.91	0.82	0.62
hexenal	0.08	0.06	0.03	0.10
$\beta-$pinene	14.29	6.45	18.17	18.04
sabinene	0.47	0.23	0.19	0.12
Δ^3-carene	0.02	0.01	0.01	0.02
myrcene+ α-phellandrene	0.47	0.59	0.61	0.88
$\alpha-$terpinene	0.02	0.01	0.01	0.02
limonene	0.60	0.68	0.82	0.84
$\beta-$phellandrene	0.43	0.46	0.58	0.76
2-pentylfuran	0.04	0.02	0.02	0.04
(Z)- β-ocimene	0.01	0.01	0.01	0.02
$\gamma-$terpinene	0.03	0.03	0.02	0.03
(E)- β-ocimene	0.34	0.36	0.33	0.85
P-cymene	0.17	0.09	0.07	0.05
terpinolene	0.10	0.08	0.08	0.14
campholene aldehyde	0.07	0.10	0.06	0.07
$\alpha-$copaene	0.17	0.23	0.14	0.16
linalool	0.07	0.05	0.10	0.05
linalyl acetate	0.57	0.36	0.35	0.14
longifolene	0.27	0.13	0.42	0.22
bornyl acetate	0.68	0.48	0.68	—
$\beta-$elemene	0.03	0.03	0.03	—
$\beta-$caryophyllene	6.73	7.53	7.11	7.65
hexadecane	0.01	trace	trace	0.03
myrtenal	0.86	0.55	0.48	0.20
trans-pinocarveol	1.44	0.97	0.95	0.29
$\alpha-$humulene	0.41	1.51	1.54	1.28
$\alpha-$terpineol	1.07	1.25	1.02	0.38
germacrene isomer	0.98	2.73	1.21	1.36
$\alpha-$terpinyl acetate	0.98	1.00	0.97	0.75
germacrene- D	13.41	16.74	18.29	10.62
$\alpha-$muurolene	1.72	1.11	1.13	0.71
$\delta-$cadinene	2.82	5.87	2.33	3.14
dodecanol	0.39	1.09	0.95	0.71
Caryophyllene oxide	0.29	0.12	0.07	0.12
Methyl eugenol	0.30	0.21	0.33	0.57
trans-methylisoeugenol	0.30	0.29	0.12	0.40
T-muurolol	0.45	0.59	0.49	0.35
farnesyl acetate	0.09	0.29	0.10	0.17
TOTAL	81.77	86.52	91.07	87.51

Trace: <0.01%

Table 9. Results of analysis of *P. nigra* needle oils from Sinop.

Collection Dates	4.8.1994	7.11.1994	20.2.1995	25.5.1995
Compounds	%	%	%	%
tricyclene	0.05	0.16	0.08	0.04
$\alpha-$pinene	24.15	40.89	36.73	43.01
camphene	0.67	1.40	0.92	0.64
hexenal	0.02	0.08	0.07	0.13
$\beta-$pinene	11.79	11.02	15.26	20.19
sabinene	0.30	1.03	0.55	0.08
Δ^3-carene	0.01	0.01	—	—-
myrcene + α-phellandrene	0.43	0.47	0.77	1.04
$\alpha-$terpinene	0.01	0.02	0.03	0.01
limonene	0.65	0.91	0.65	0.91
$\beta-$phellandrene	0.49	0.35	0.12	0.94
2-pentylfuran	0.04	0.03	0.03	0.04
(Z)- β-ocimene	0.01	—	—	0.02
$\gamma-$terpinene	0.03	0.02	0.02	0.02
(E)- β-ocimene	0.51	0.25	0.68	1.09
P-cymene	0.10	0.28	0.18	0.03
terpinolene	0.09	0.07	0.09	0.11
campholene aldehyde	0.09	0.02	0.04	0.07
$\alpha-$copaene	0.23	0.45	trace	0.19
linalool	0.02	0.09	0.05	0.01
linalyl acetate	0.81	0.93	0,63	0.07
longifolene	0.35	0.33	0.28	0.14
bornyl acetate	0.32	0.58	0.42	0.13
$\beta-$elemene	0.05	0.04	0.03	0.01
$\beta-$caryophyllene	8.09	4.33	6.74	8.27
hexadecane	0.08	0.09	0.07	0.02
myrtenal	1.29	1.33	0.86	0.10
trans-pinocarveol	1.39	2.29	1.35	0.17
$\alpha-$humulene	1.46	1.31	1.45	1.25
$\alpha-$terpineol	3.20	5.09	3.37	0.24
germacrene isomer	1.58	0.48	0.94	1.37
$\alpha-$terpinyl acetate	0.40	4.43	0.77	0.22
germacrene- D	8.22	1.81	6.47	10.89
$\alpha-$muurolene	0.95	2.43	1.83	0.18
$\delta-$cadinene	2.67	0.78	1.54	2.39
dodecanol	5.38	1.93	1.46	0.41
Caryophyllene oxide	0.11	0.10	0.07	0.05
Methyl eugenol	0.27	0.41	0.43	0.29
trans-methyl isoeugenol	0.37	0.21	0.16	0.09
T-muurolol	0.71	0.18	0.20	0.16
farnesyl acetate	0.40	0.05	0.07	0.06
TOTAL	77.79	86.60	85.41	95.08

Trace: <0.01%

Table 10. Results of analysis of *P. nigra* needle oils from Samsun.

Collection Dates	25.5.1994	18.8.1994	17.11.1994	17.2.1995
Compounds	%	%	%	%
tricyclene	0.20	0.25	0.30	0.19
$\alpha-$pinene	24.49	33.74	30.44	30.58
camphene	1.08	1.17	1.42	0.88
hexenal	0.07	0.03	0.16	0.17
$\beta-$pinene	5.01	1.15	9.44	14.49
sabinene	0.54	0.19	0.66	0.22
Δ^3-carene	0.02	0.01	0.02	0.02
myrcene + α-phellandrene	0.43	0.43	0.46	0.67
$\alpha-$terpinene	0.02	trace	0.02	0.04
limonene	0.57	0.45	0.68	0.67
$\beta-$phellandrene	0.25	0.15	0.41	0.52
2-pentylfuran	0.04	0.04	0.10	0.05
(Z)- β-ocimene	0.02	0.03	0.01	0.02
$\gamma-$terpinene	0.03	0.02	0.04	0.07
(E)- β-ocimene	0.38	0.88	0.51	0.75
P-cymene	0.14	0.08	0.23	0.11
terpinolene	0.15	0.07	0.08	0.38
campholene aldehyde	0.14	0.21	0.10	0.12
$\alpha-$copaene	0.23	0.46	0.20	0.28
linalool	0.09	0.17	0.08	0.19
linalyl acetate	0.80	0.13	0.63	0.45
longifolene	0.16	0.88	0.23	0.43
bornyl acetate	0.70	0.69	1.05	0.22
$\beta-$elemene	0.06	0.03	0.03	0.05
$\beta-$caryophyllene	6.90	13.31	6.29	8.05
hexadecane	0.12	0.09	0.20	0.15
myrtenal	1.26	0.19	0.81	0.84
trans-pinocarveol	1.45	0.34	1.72	0.89
$\alpha-$humulene	1.32	2.25	1.73	1.56
$\alpha-$terpineol	2.10	3.49	1.92	1.91
germacrene isomer	1.36	0.62	1.92	0.84
$\alpha-$terpinyl acetate	1.04	1.02	1.02	1.07
germacrene- D	9.67	13.37	9.87	9.22
$\alpha-$muurolene	1.23	1.15	2.01	1.09
$\delta-$cadinene	3.60	6.25	3.31	3.81
dodecanol	3.52	3.16	2.11	1.12
Caryophyllene oxide	0.41	0.03	0.40	0.16
Methyl eugenol	0.27	0.06	0.32	0.57
trans-methyl isoeugenol	0.52	0.26	0.19	0.96
T-muurolol	0.66	0.13	0.46	0.43
farnesyl acetate	0.45	0.12	0.16	0.15
TOTAL	71.50	87.10	81.74	84.39

Trace: <0.01%

Table 11. The contents of major 4 constituents in the pine oils.

Pinus nigra	$\alpha-$pinene	$\beta-$pinene	germacrene-D	$\beta-$caryophyllene
Antalya	24.40-44.16	14.05-21.89	7.97-18.03	6.40-8.42
Denizli	4.51-49.63	1.42-13.07	11.23-18 43	5.87-21.48
Kastamonu	21.31-35.53	6.45-18.17	10.62-18.29	6.73-7.65
İçel	22.20-43.05	22.40-34.10	6.45-14.91	5.65-9.21
Samsun	24.49-33.74	1.15-14.49	9.22-13.37	6.29-13.31
Bursa	35.94-40.87	3.11-19.52	13.07-21.38	6.34-8.76
Kütahya	24.69-45.09	10.22-17.63	11.27-15.43	5.26-10.19
Sinop	24.15-43.01	11.02-20.19	1.81-10. 89	4.33-8.27

Several chemotaxonomic studies on the essential oil composition of *P. nigra* have been published.[32,36,37] According to Roussis,[38] *P. nigra* growing in Greece belongs to the chemotype C and the essential oil content of the needles was found in the following order: α-pinene > germacrene D > limonene > β-pinene

Kubeczka et al. reported α-pinene, germacrene D, β-pinene, and limonene as major constituents in the oils of German materials.[39]

Our results suggested the following order for the Turkish *P. nigra* oils:

$\alpha-$pinene > β-pinene > germacrene D > β-caryophyllene

The Turkish pine needle oils may be characterized by high amounts of β- caryophyllene, germacrene D, Δ^3 cadinene, and α-terpinyl acetate. Although Turkish oils contained low contents of Δ^3 carene, some of the oils were found to contain about 1% humulene, α-terpinolene, and α-muurolene, depending upon their site of collection.

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