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STUDY OF THE ESSENTIAL OIL COMPOSITION OF *Pinus sylvestris* FROM TURKEY

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The needle oils of Pinus sylvestris *L. were analyzed by GC and GC-MS. The results showed some qualitative and quantitative variations. Forty-three components were identified in the oils of* P. sylvestris. *All the samples of essential oils contained* α*-pinene, camphene, and* β*-pinene as major constituents. Chemical variations of* P. sylvestris *samples were discussed.*

Key words: *Pinus sylvestris* L., essential oils, α-pinene, germacrene-D, β-pinene, β-caryophyllene.

Pinus sylvestris is distributed mainly in North Anatolia, throughout Europe, extending into Caucasia. Five *Pinus* species are recorded in Turkey (*Pinus brutia, nigra, sylvestris, pinea, halepensis*) and three of them (*Pinus brutia, nigra*, *sylvestris*) are commercially utilized. Previous studies on *Pinus* species in Turkey were mainly on improving the yield of turpentine production. Pine oils are widely used as fragrances in cosmetics, flavoring additives for food and beverages, scenting agents in a variety of household products, and intermediates in the synthesis of perfume chemicals.

They are also used for medicinal purposes in aromatherapy as carminative, rubefacient, emmenagogue, and abortifacient agents. *Pines* are amoung the most important forest trees in the Mediterranean region. There are numerous studies dealing with the essential oils of conifer species and especially those of *Pinus*. These studies deal with the reasons for the chemical variation of pine oils from the geographical, seasonal, genotypic, and environmental points of view [1–9]. The present study reports on the variations of essential oil yield and composition in the leaves of *P. sylvestris* collected from different parts of Turkey.

The 43 compounds identified in the oils are listed in Tables 1, 2.

The essential oil content in *P. sylvestris* ranged between 0.22–0.82%. In Samsun and Kutahya samples, the highest volatile oil contents were found in summer. In Sinop and Kastamonu, the ratio was higher in spring and continued to stay high during the summer season. On the other hand, the volatile oil content was found to be higher in fall than those in winter and spring in Kutahya and Samsun materials.

The main constituents in *P. sylvestris* needle oils were α-pinene, camphene, and β-pinene.

The α-pinene, β-pinene, and camphene ratios in essential oil samples ranged between 19.44–56.88%, 2.87–17.09%, and 0.44–16.84% respectively. α-Pinene and β-pinene ratios in Sinop samples were generally found to be lower (22–55%). This ratio was higher in other study areas (40–60%). Δ^3-Carene contents were low in all the samples. Although it is not possible to make concrete conclusions, the tricyclene, camphene, Δ-cadinene, germacrene-D, and caryophyllene contents in all the samples were similar. In addition, the amounts of *trans*-verbenol, limonene, (*E*)-β-ocimene, etc. were around 1%.

Chemotypic variations of the essential oils of *Pinus sylvestris* have previously been reported [10–16]. Previous studies have concluded that there are pinene and carene groups, and another third group between types was also determined.

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TABLE 1. Results of Analysis of *P. sylvestris* Needle Oils from Kutahya and Kastamonu (Collection sites, %)

Compound	Kutahya				Kastamonu			
	21.09.94 (0.53), %	27.12.94 (0.27), %	02.04.95 (0.55), %	28.07.95 (0.82), %	08.08.94 (0.38), %	11.11.94 (0.27), %	05.02.95 (0.32), %	29.05.95 (0.43), %
Tricyclene	1.27	0.98	1.47	1.38	2.18	2.63	2.50	1.56
α-Pinene	**39.72**	**43.04**	**48.04**	**47.73**	**51.09**	**36.21**	**41.06**	**56.88**
Camphene	**6.37**	**4.78**	**6.53**	**5.52**	**8.43**	**10.73**	**9.77**	**6.81**
Hexenal	0.02	0.04	0.10	0.13	0.21	0.33	0.20	0.08
β-Pinene	**6.00**	**17.09**	**11.70**	**10.04**	**5.54**	**7.03**	**6.66**	**11.19**
Sabinene	0.18	0.20	0.07	0.12	0.50	0.72	0.76	0.17
Δ^3-Carene	Tr.	0.01	0.01	0.01	-	0.01	0.02	0.02
Myrcene	2.47	2.66	2.41	1.89	1.33	0.67	1.08	2.17
Limonene	0.75	4.22	1.80	1.19	0.84	0.87	0.71	0.82
β-Phellandrene	0.65	0.64	0.59	0.49	0.27	0.26	0.32	0.48
cis-β-Mentha-6,8-dien-2-ol	0.06	0.09	0.08	0.06	0.15	0.18	0.21	0.04
2-Pentylfuran	0.05	0.13	0.09	0.08	0.07	0.09	0.09	0.03
γ-Terpinene	0.01	0.08	0.09	0.07	0.07	0.08	0.06	0.07
(*E*)-β-Ocimene	2.68	2.86	2.36	1.99	0.48	0.38	0.93	0.77
p-Cymene	0.11	0.13	0.13	0.07	0.22	0.45	0.36	0.08
Terpinolene	0.11	0.16	0.12	0.09	0.20	0.12	0.17	0.17
Tetradecane	0.01	0.03	0.02	0.01	0.05	0.11	0.07	0.01
β-Pinene oxide	0.04	0.09	0.10	0.05	0.12	0.23	0.16	0.06
Camphenilone	0.03	0.03	0.04	0.02	0.04	0.07	0.10	0.01
Campholene aldehyde	0.37	0.46	0.45	0.17	0.67	1.01	0.06	0.24
α-Copaene	0.16	0.11	0.07	0.03	0.12	0.13	0.44	0.06
Camphor+pinocamphor	0.22	0.13	0.16	0.20	0.16	0.17	0.37	0.09
Benzaldehyde+β-bourbonene	0.05	0.07	0.04	0.11	0.12	0.17	0.16	0.02
Pinocarvone	0.23	0.37	0.22	0.11	0.31	0.47	0.50	0.19
Bornyl acetate	0.44	0.11	0.19	0.11	1.18	2.27	3.92	0.08
β-Caryophyllene+terpinen-4-ol	4.34	2.28	2.42	4.82	1.01	0.78	0.57	1.34
Myrtenal	0.47	0.49	0.40	0.20	0.52	0.94	0.89	0.25
trans-Pinocarveol	0.68	1.01	0.72	0.37	0.78	1.14	1.34	0.35
cis-Verbenol	0.37	0.11	0.29	0.11	0.52	0.61	0.92	0.17
p-Mentha-1,5-dien-8-ol	1.07	0.63	0.70	1.03	0.68	0.73	0.84	0.31
trans-Verbenol	1.50	0.26	0.94	0.41	2.01	2.46	3.53	0.71
Germacrene-D	3.87	1.34	1.73	4.11	0.71	0.65	0.32	1.00
Δ-Cadinene	3.49	1.32	1.74	3.81	1.71	1.25	0.57	1.98
Myrtenol	0.49	0.62	0.47	0.33	0.45	0.80	0.55	0.22
trans-Carveol	0.24	0.21	0.20	0.11	0.32	0.48	0.56	0.10
p-Cymen-8-ol	0.11	0.06	0.04	Tr.	0.02	0.03	0.04	0.01
Caryophyllene oxide	1.42	1.29	1.11	0.70	1.29	1.29	1.45	0.63
Spathulenol	0.61	0.53	0.85	0.58	1.62	3.50	1.07	1.23
Torreyol	0.61	0.27	0.29	0.28	0.37	0.21	0.22	0.40
T-Muurolol	0.69	0.63	0.61	0.53	0.51	0.35	0.46	0.59
Total	81.96	89.56	89.39	89.06	86.87	80.61	84.01	91.39

Tr.: trace <0.01%.

TABLE 2. Results of Analysis of *P. sylvestris* Needle Oils from Sinop and Samsun (Collection sites, %)

Compound	Sinop				Samsun			
	04.08.94 (0.33), %	07.11.94 (0.28), %	20.02.95 (0.38), %	25.05.95 (0.48), %	18.08.94 (0.70), %	17.11.94 (0.54), %	17.02.95 (0.22), %	25.05.95 (0.49), %
Tricyclene	2.77	3.28	4.29	1.64	3.04	2.42	0.10	1.40
α-Pinene	**19.44**	**28.06**	**24.20**	**44.12**	**43.44**	**39.95**	**33.87**	**44.30**
Camphene	**10.75**	**15.25**	**16.84**	**10.31**	**13.10**	**9.83**	**0.44**	**5.50**
Hexenal	0.06	0.13	0.26	0.11	0.05	0.15	0.08	0.10
β-Pinene	**3.69**	**3.69**	**2.87**	**11.35**	**5.17**	**7.12**	**11.19**	**15.76**
Sabinene	0.80	0.98	0.90	0.06	0.16	0.41	0.10	0.24
Δ^3-Carene	Tr.	1.14	1.41	0.01	-	0.01	0.02	-
Myrcene	0.76	0.76	0.66	3.36	2.72	1.07	0.77	1.29
Limonene	0.76	0.97	0.51	2.28	0.87	0.42	0.65	0.64
β-Phellandrene	0.19	0.16	0.13	0.70	0.39	0.23	Tr.	0.59
cis-β-Mentha-6,8-dien-2-ol	0.20	0.23	0.22	0.07	0.04	0.11	0.60	0.05
2-Pentylfuran	0.07	0.05	0.07	0.17	0.02	0.05	0.05	0.04
γ-Terpinene	0.09	0.10	0.20	0.17	0.10	0.10	0.03	0.05
(*E*)-β-Ocimene	0.51	0.29	0.59	3.35	1.30	0.45	0.21	0.65
p-Cymene	0.45	0.50	0.90	0.08	0.13	0.21	0.05	0.10
Terpinolene	0.09	0.14	0.14	0.22	0.21	0.07	0.08	0.14
Tetradecane	0.14	0.17	0.08	0.08	0.02	0.03	-	-
β-Pinene oxide	0.28	0.30	0.15	0.08	0.08	0.13	0.04	0.07
Camphenilone	0.23	0.18	0.23	0.01	0.02	0.06	0.36	0.02
Campholene aldehyde	Tr.	1.32	1.13	0.20	0.36	0.55	0.17	0.31
α-Copaene	1.69	0.52	0.65	0.08	0.04	0.05	0.02	0.06
Camphor+pinocamphor	0.71	0.31	0.31	0.29	0.13	0.21	0.20	0.14
Benzaldehyde+β-bourbonene	0.52	0.38	0.36	Tr.	0.05	0.13	-	0.05
Pinocarvone	1.00	0.69	0.53	0.13	0.17	0.30	0.46	0.24
Bornyl acetate	0.79	2.54	0.39	0.31	0.53	0.21	0.14	0.23
β-Caryophyllene+terpinen-4-ol	0.73	0.56	0.69	1.53	2.07	5.97	9.32	3.81
Myrtenal	1.82	1.19	1.12	1.21	0.24	0.61	0.16	0.39
trans-Pinocarveol	2.20	1.88	1.50	0.29	0.50	0.85	0.26	0.58
cis-Verbenol	1.82	1.18	0.89	0.05	0.27	0.29	0.09	0.30
p-Mentha-1,5-dien-8-ol	0.80	1.06	0.86	0.42	0.44	1.47	1.59	0.77
trans-Verbenol	9.61	5.07	3.41	0.23	1.19	1.17	0.65	1.21
Germacrene-D	0.28	0.16	0.28	0.91	2.67	2.10	10.30	5.01
Δ-Cadinene	1.33	0.72	1.23	2.06	4.73	3.68	7.28	2.97
Myrtenol	1.00	0.83	0.75	0.21	0.31	0.53	0.48	0.37
trans-Carveol	1.05	0.96	0.71	0.12	0.19	0.32	0.07	0.16
p-Cymen-8-ol	0.05	0.10	0.53	0.04	0.03	0.16	0.03	Tr.
Caryophyllene oxide	2.90	1.39	2.03	0.42	0.79	2.23	0.54	0.72
Spathulenol	2.04	1.71	1.30	1.26	1.41	1.32	0.08	0.26
Torreyol	0.59	0.25	0.44	0.47	0.57	0.26	0.19	0.17
T-Muurolol	1.15	0.41	0.71	0.88	1.40	0.42	0.38	0.39
Total	73.36	79.59	74.47	89.28	89.13	85.65	81.05	89.08

Tr.: trace <0.01%.

TABLE 3. Comparison of Chemical Composition of Volatile Oils from *P. sylvestris* in Turkey, Russia, Portugal, France, Finland and Germany

Compounds	Countries					
	Turkey	Russia	Portugal	France	Finland	Germany
Tricyclene	+	+	(-)	+	+	+
α-Pinene	+	+	+	+	+	+
Camphene	+	+	+	+	+	+
Hexenal	+	(-)	(-)	(-)	(-)	(-)
β-Pinene	+	+	+	+	+	+
Sabinene	+	(-)	+	+	+	+
Δ^3-Carene	+	+	+	+	+	+
Myrcene	+	(-)	(-)	+	+	+
Limonene	+	+	+	+	+	+
β-Phellandrene	+	+	(-)	+	+	+
cis-β-Mentha-6,8-dien-2-ol	+	(-)	(-)	(-)	(-)	(-)
2-Pentylfuran	+	(-)	(-)	(-)	(-)	(-)
γ-Terpinene	+	+	+	+	+	+
(*E*)-β-Ocimene	+	(-)	+	+	+	+
p-Cymene	+	(-)	(-)	+	+	+
Terpinolene	+	(-)	+	+	+	+
Tetradecane	+	(-)	(-)	(-)	(-)	(-)
β-Pinene oxide	+	(-)	(-)	(-)	(-)	(-)
Camphenilone	+	(-)	(-)	(-)	(-)	(-)
Campholene aldehyde	+	(-)	(-)	(-)	(-)	(-)
α-Copaene	+	(-)	(-)	+	(-)	+
Camphor+pinocamphor	+	(-)	(-)	(-)	(-)	(-)
Benzaldehyde	+	(-)	(-)	(-)	(-)	(-)
β-Bourbonene	+	(-)	(-)	(-)	(-)	(-)
Pinocarvone	+	(-)	(-)	(-)	(-)	(-)
Bornyl acetate	+	+	+	+	(-)	+
β-Caryophyllene	+	+	(-)	+	(-)	+
Terpinene-4-ol	+	(-)	+	+	(-)	(-)
Myrtenal	+	(-)	(-)	(-)	(-)	(-)
trans-Pinocarveol	+	(-)	(-)	(-)	(-)	(-)
cis-Verbenol	+	(-)	(-)	(-)	(-)	(-)
p-Mentha-1,5-dien-8-ol	+	(-)	(-)	(-)	(-)	(-)
trans-Verbenol	+	(-)	(-)	(-)	(-)	(-)
Germacrene-D	+	(-)	+	(-)	(-)	(-)
Δ-Cadinene	+	(-)	(-)	+	(-)	(-)
Myrtenol	+	+	(-)	(-)	(-)	(-)
trans-Carveol	+	(-)	(-)	(-)	(-)	(-)
p-Cymen-8-ol	+	(-)	(-)	(-)	(-)	(-)
Caryophyllene oxide	+	(-)	(-)	(-)	(-)	(-)
Spathulenol	+	(-)	(-)	(-)	(-)	(-)
Torreyol	+	(-)	(-)	(-)	(-)	(-)
T-Muurolol	(-)	(-)	(-)	+	(-)	+
α-Terpinene	(-)	+	+	+	+	+
1,8-Cineole	(-)	+	(-)	(-)	(-)	(-)
n-Cymol	(-)	+	(-)	(-)	(-)	(-)
Citrate (geranial)	(-)	+	(-)	(-)	(-)	(-)
Citrate (neral)	(-)	+	(-)	(-)	(-)	(-)
Longifolene	(-)	+	+	+	(-)	(-)
Humulene	(-)	+	+	+	(-)	+
Terpineol	(-)	+	+	+	(-)	+
α-Muurolene	(-)	+	+	+	(-)	(-)
γ-Cadinene	(-)	+	+	+	(-)	(-)

TABLE 3. (continued)

Compounds	Countries					
	Turkey	Russia	Portugal	France	Finland	Germany
Chamazulene	(-)	+	(-)	(-)	(-)	(-)
β-Bisabolene	(-)	+	(-)	(-)	(-)	(-)
α-Curcumene	(-)	+	(-)	(-)	(-)	(-)
Santene	(-)	(-)	+	+	(-)	(-)
(*Z*)-β-Ocimene	(-)	(-)	+	(-)	+	(-)
Linalool	(-)	(-)	+	(-)	(-)	(-)
Phenylalcohol	(-)	(-)	+	(-)	(-)	(-)
Pinocarveol	+	(-)	+	+	(-)	(-)
Borneol	(-)	(-)	+	(-)	(-)	(-)
Nerol	(-)	(-)	+	(-)	(-)	(-)
Geraniol	(-)	(-)	+	(-)	(-)	(-)
Linalyl acetate	(-)	(-)	+	(-)	(-)	(-)
Piperitone	(-)	(-)	+	(-)	(-)	(-)
Nerolidol	(-)	(-)	+	+	(-)	(-)
Δ-Elemene	(-)	(-)	(-)	+	(-)	(-)
α-Ylangene	(-)	(-)	(-)	+	(-)	(-)
β-Guaiene	(-)	(-)	(-)	+	(-)	(-)
β-Farnesene	(-)	(-)	(-)	+	(-)	(-)
γ-Muurolene	(-)	(-)	(-)	+	(-)	(-)
γ-Patcoulene	(-)	(-)	(-)	+	(-)	(-)
4,10-Dimethyl-7-isopropyl-(4,4,0) bicyclo-1,4-decadiene	(-)	(-)	(-)	+	(-)	(-)
α-Cadinene	(-)	(-)	(-)	+	(-)	(-)
Calamenene	(-)	(-)	(-)	+	(-)	(-)
T-Cadinol	(-)	(-)	(-)	+	(-)	+
Δ-Cadinol	(-)	(-)	(-)	+	(-)	(-)
α-Cadinol	(-)	(-)	(-)	+	(-)	+
β-Fenchene	(-)	(-)	(-)	(-)	(-)	(-)
α-Cubenene	(-)	(-)	(-)	(-)	(-)	+
Germacre-1(10)-E-SE-dien-4-ol	(-)	(-)	(-)	(-)	(-)	+

For instance (Table 3): *P. sylvestris* growing in Spain belongs to the pinene chemotype (69.5% α-pinene), *P. sylvestris* growing in different regions in Russia belongs to the pinene chemotype (30.6–71.8% α-pinene), *P. sylvestris* growing in Portugal belongs to the pinene chemotype (29.32% α-pinene), *P. sylvestris* growing in Puy-de-dome region in France belongs to the carene chemotype (43.4% Δ^3-carene), Haute-Loire region in France belongs to the pinene chemotype (41.1% α-pinene), *P. sylvestris* growing in Finland belongs to the pinene chemotype (45.86% α-pinene), *P. sylvestris* growing in Germany belongs to the pinene chemotype (42.08% α-pinene), *P. sylvestris* growing in Yakutia belongs to the pinene chemotype (28% α-pinene), *P. sylvestris* growing in Lithuania belongs to the pinene chemotype (18.5–33% α-pinene), and *P. sylvestris* growing in Greece belongs to the pinene chemotype (19.44% α-pinene, 17.27% β-pinene). In the light of the above information, *P. slyvestris* growing in Turkey belongs to the pinene chemotype.

EXPERIMENTAL

Plant Material and Hydrodistillation. Young needles of *Pinus sylvestris* L. were collected from different natural regions in different seasons; in Muratdagi region in Kutahya, in Kuzyaka region in Kastamonu, in Goktepe region in Sinop, and in Kertuce region in Samsun.

The needles were dried and cut into small pieces before use. Each collection was hydrodistilled using a Clevenger type apparatus. The oil was collected for 3 hours after the first drop of the distillate eluted from a cold finger. The oils were kept in a refrigerator until the GC-MS analysis. Oil yields are reported on a moisture-free basis.

GC-MS Analysis. The oils were analyzed by GC-MS using a Hewlett-Packard GC-MSD system. A Thermon 600 T column (50 m $\times$ 0.25 mm i.d. with 0.25 μm film thickness) was used with helium as carrier gas. GC oven temperature was kept at 70°C for 10 min and programmed to 180° C at a rate of 2°C/min, 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 70 eV. Mass range was from *m/z* 10–400. 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 TIC by the computer.

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