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A Comparative Study on Turpentine Oils of Oleoresins of *Pinus sylvestris* L. from Three Districts of Denizli

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Abstract: Oleoresin samples collected from *Pinus sylvestris* L. trees from Acıpayam, Çal and Çamlıbel, three different locations in Denizli-Turkey. The constituents of the turpentine oil, obtained by hydrodistillation of oleoresin of *Pinus sylvestris* L., were identified by GC-MS. Fifty four constituents were detected from the turpentine oil, which constituted about between 96.2% and 98.2% of the total amount. Major constituents of the oil were α-pinene, β-pinene, camphene, longifolene, Δ^3-carene, limonene and β-caryophyllene.

Keywords: Oleoresin; turpentine; GC-MS; *Pinus sylvestris*; essential oil; α-pinene; β-pinene; *β*-caryophyllene.

1. Introduction

Named as the "spirits of turpentine", "pine tree terpenic", "pine oleoresin", "gum turpentine", "terpenes oil" or "turpentine from Bordeaux" is the term "essential oil of turpentine" which designates the terpenic oil, obtained by hydrodistillation of the gum pine. The terpenic oil is used in the pharmaceutical industry, perfume industry, food additives and other chemical industries (household cleaning products, paintings, varnishes, rubber, insecticides, etc) due to its pleasant fragrance [1].

The terpenic oil was used by the eminent doctors of antiquity, Hippocrates, Galen or Dioscorides for its properties that helped acting against lung diseases and biliary lithiasis. It was recommended against blennorrhoea and cystitis in France by Thillenius, Pitcairn, Récamier and Martinet. It was prescribed against neuralgias by Chaumeton, Peschiez, Kennedi, and Mérat. The treatment of rheumatism, sciatica, nephritis, drop, constipation and mercury salivation also required terpenic oil.

Anti-parasitic, analgesic, revulsive, disinfectant (external use); balsamic, active on bronchial secretion as well as on pulmonary and genito-urinary tract infections, haemostatic, active on dissolving gallstones, diuretic, antispasmodic, antirheumatic, de-worming, active as an antidote for phosphorous poisonings and active on improving the ciliary and the secretory activity in patients who

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present chronic obstructive bronchitis (internal use) were several characteristics that were attributed to terpenic oil which were reported in modern phytotherapy [1-3].

Pine exists with five species that make it the dominating conifer of Turkey. *Pinus brutia* Ten. grows extensively in the Mediterranean and the Aegean region, Marmara region, and also in some localities of the coasts of the western Black Sea region in a total area of about 3 mha. (Anon 1987) is represented in the broadest habitat. Also another dominating pine species is *P. nigra* Arn., which inhabits the inner regions of northern Anatolia, the Aegean and the Mediterranean districts, covering more than 2 mha. The northern parts of Anatolia is the habitat of *P. sylvestris* L. The Aegean region is the habitat of *P. pinea* L. which has a limited distribution. A limited area of the eastern Mediterranean region is the survival district of *P. halepensis* Mill. [4,5].

Pine oleoresin, which is traditionally obtained by tapping the bark (bark chipping) of the pine tree and collection of the resulting exudate, is an important forestry product. Complex mixtures of acidic and neutral diterpenes together with a more or less important fraction of volatile compounds (mono and sesquiterpenes) compose the oleoresins. Steam distillation is used in the industry to convert by steam distillation into gum turpentine (volatile compounds) and gum rosin (diterpenes). Later, food gums, adhesives, coatings, printing inks, disinfectants, cleaners, pharmaceuticals, fragrances and flavoring industries use both gums to turn them into chemical industrial products [6].

The characterization of the pine oleoresin is the research topic of several studies. Neutral monoterpenes (mostly α-pinene) and diterpene acids are the main components. Although not the major components, neutral diterpenes and sesquiterpenes are also represented in appreciable to moderate amounts. Depending on the pine species and the geographical origins [6,7-18]., bicyclic diterpenes (labdanes) [19], tricyclic diterpenes (abietanes and pimaranes) and macrocyclic diterpenes (cembranes) were reported among the main components. *Pinus* species in Turkey were mainly on improving the yield of turpentine production [20]. Also there are some working about turpentine on *Pinus* species in Turkey [21-23].

2. Materials and Methods

2.1. Plant Material

The oleoresin was collected from three different individual *Pinus sylvestris* L. trees from Denizli in Turkey. The trees were taken from Acıpayam (A), Çal (B) and Çameli (C) locations, respectively. Each pine tree approximately has a same age and diameter. Oleoresin was collected about 250 g from each pine tree.

2.2. Isolation of the Turpentine Oil and GC-MS analysis

The turpentine oil was extracted from the 50 g resin by hydrodistillation with glycerin in a Clevenger apparatus for 4 h. The obtained turpentine oil was subsequently dried over anhydrous Na_2SO_4 and stored under refrigeration until analyzed and tested [24]. Turpentine oil yields were mL/100g [25,26] and were kept in a refrigerator until the GC-MS analysis. Turpentine oil yields reported per dry material and oil results given in w/w, calculated on freeze-dried sample [27].

The hydrodistilled samples include turpentine oil were analysed using an HP 6890-5973 GC-MS instrument equipped with an HP-5 capillary column (25 m / 0.25 mm i.d., 0.11 μm film thickness). Helium was used as the carrier gas at 0.8 mL/min flow rate. The injection volume was 0.5 μl with a split ratio of 1:50. The column oven was temperature programmed starting from 50 °C (0.5 min) to 250 °C, at 4 °C/min heating rate. After 10 min of hold time at 250 °C the temperature program was continued at 10 °C/min to 290 °C for 15 min. The split-injector and MS-transfer line were held at 260 °C and 280 °C, respectively. The MS was operated in electron impact ionisation mode at 70 eV electron energy. Mass range was from m/z 35 to 400 amu. Compound identification was based on

mass spectra, referring to NIST98 and WILEY275 mass spectral libraries, and also comparing measured retention index (RI) values of components with literature data [28]. The quantitative area-percent measurements were based on peak-areas from the GC-MS total ion current (TIC) data.

3. Results and Discussion

Turpentine oil yields of resin from *Pinus sylvetris* L. trees were given as both volumetric and gravimetric results in Table 1. According to the table, the highest volumetric (5.6%) and gravimetric (4.72%) turpentine was obtained from resin C. Moreover, the lowest turpentine was obtained from resin A as 3.96 %, and 3.28% , respectively.

When comparing the physical properties of turpentine essential oils, the color of turpentine obtained from resin B was clear light yellow. Turpentine A (from Acıpayam) was also light, but lighter than turpentine B (from Çal). Moreover, turpentine C (from Çameli) was even lighter than those of turpentine A and B, and close to white.

Table 1. Turpentine Oil Yields (%).

Oil Yields (%)			
Sample Site	Volumetric (mL/100 g)	Gravimetric (w/w)	Oil Physical Properties (Colour)
Acıpayam	3.96	3.28	Light and Straw Yellow
Çal	4.36	3.70	Light and Straw Yellow
Çameli	5.60	4.72	Canary and Matt

The chemical compositions of the turpentine oils are summarized in Table 2. 98.18%, 95.57% and 96.24% of turpentine of pine resins obtained from Acıpayam, Çal and Çameli regions of Turkey, respectively. As can be seen from Table 2, α-pinene was the dominant compound ranging from %34.99 to 43.60%, followed by β-pinene, Δ^3-carene, longifolene, cymene, limonene, trans-pinocarveol and β-caryophyllene.

Comparison of the turpentines, obtained from different locations exhibited some differences at the percentage of their chemical constituents; the highest amount of α-pinene was obtained from turpentine A (from Acıpayam) with a percentage of 43.60% while it was obtained from turpentine B (from Çal) with a lowest percentage (34.99%) indicating that not a big difference. The highest amount of β-pinene, 3-carene and limonene were found in Turpentine C (from Çameli) as 29.40%, 9.68% and 2.07%, respectively while these monoterpenes was found in turpentine B in lowest amount as 15.29%, 6.54 % and 1.71 %, respectively. In contrast, highest α-longifolene and trans-pinocarveol were observed in turpentine B as 9.59% and 2.83% while they were detected in the lowest amounts in turpentine C as 4.39%; 0.85%. Sesquiterpene β-caryophyllene was found in turpentine C with highest percentage (1.65%) while it was found at the lowest amonut in turpentine A (1.15%). Interestingly carvone and α-fenchene were not observed in turpentine C, not even in trace amounts.

The identification of constituents showed that the presence of monoterpene hydrocarbons, monoterpene alcohols, sesquiterpene hydrocarbons and oxygenated terpenes in the turpentines.

Table 2. Percent composition of components in turpentine oil (%).

No	RI	Compounds	% (w/w) Acıpayam (A)	% (w/w) Çal (B)	% (w/w) Çameli (C)	Identification MS, RI
1	924	tricyclene	0.13	0.12	0.05	MS, RI
2	926	α-thujene	0.11	0.14	0.12	MS, RI
3	936	α-pinene	43.60	34.99	42.82	MS, RI
4	948	α-fenchene	0.14	0.18	-	MS, RI
5	949	camphene	2.20	2.13	1.00	MS, RI
6	954	thuja-2,4(10)-diene	0.81	1.04	0.36	MS, RI
7	963	3,7,7-trimethyl-cyclohepta-1,3,5-triene	1.12	1.16	0.36	MS
8	978	β-pinene	17.06	15.29	29.40	MS, RI
9	989	β-myrcene	0.30	0.24	0.47	MS, RI
10	1010	3-carene	7.69	6.54	9.68	MS, RI
11	1019	α-terpinene	0.07	0.12	0.06	MS, RI
12	1021	p-cymene	0.20	0.21	0.04	MS, RI
13	1023	o-cymene	1.47	1.54	0.47	MS, RI
14	1026	limonene	1.77	1.71	2.07	MS, RI
15	1056	γ-terpinene	0.14	0.18	0.12	MS, RI
16	1081	m-cymenene	0.23	0.37	0.05	MS, RI
17	1087	α-terpinolene	0.19	0.21	0.39	MS, RI
18	1088	p-cymenene	0.52	0.76	0.12	MS, RI
19	1113	α-fenchol	0.39	0.53	0.09	MS, RI
20	1123	α-campholene aldehyde	0.76	1.03	0.28	MS, RI
21	1133	nopinone	-	0.26	0.01	MS, RI
22	1135	trans-pinocarveol	1.95	2.83	0.85	MS, RI
23	1139	camphor	0.11	0.14	0.02	MS, RI
24	1142	exo-methyl-camphenilol	0.24	0.25	0.11	MS
25	1156	trans-3-pinanone	0.16	0.20	0.05	MS, RI
26	1158	pinocarvone	0.48	0.75	0.18	MS, RI
27	1162	borneol	0.43	0.75	0.08	MS, RI
28	1165	p-mentha-1,5-dien-8-ol	0.53	1.00	0.17	MS, RI
29	1177	4-terpineol	0.27	0.40	0.15	MS, RI
30	1187	Cumyl alcohol	0.64	0.86	0.15	MS
31	1190	α-terpineol	0.55	0.93	0.35	MS, RI
32	1193	myrtenal	0.61	1.11	0.20	MS, RI
33	1195	myrtenol	0.70	1.25	0.35	MS, RI
34	1206	verbenone	0.68	1.53	0.12	MS, RI
35	1218	trans-carveol	0.15	0.39	0.04	MS, RI
36	1242	carvone	0.05	0.13	-	MS, RI
37	1348	α-longipinene	0.54	0.71	0.41	MS, RI
38	1367	cyclosativene + longicyclene	0.50	0.66	0.27	MS, RI
39	1370	α-ylangene	0.07	0.10	0.05	MS, RI
40	1374	α-copaene	0.04	0.09	0.02	MS, RI
41	1388	sativene	0.13	0.19	0.07	MS, RI
42	1403	α-longifolene	6.78	9.59	4.39	MS, RI
43	1420	β-caryophyllene	1.15	1.45	1.65	MS, RI
44	1453	α-humulene	0.17	0.26	0.23	MS, RI
45	1559	longicamphelynole	0.03	0.12	0.01	MS, RI
46	1578	caryophyllene oxide	0.33	0.98	0.26	MS, RI
47	1607	humulene oxide	0.05	0.15	0.04	MS, RI
		Total	96.24	95.57	98.18	

As a conclusion, Turpentine can be used in the many areas of industry such as medicine, pharmacy, food, cosmetics, paint and coatings, automotive and weapon industry etc. Consequently the production of resins and turpentine being economically and strategically important product of pine species grown naturally in Turkey which should be encouraged and this eventually help the local people by economical means.

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