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Geographical Variation of Essential Oil of *Pinus pinea* Needles in Turkey

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Abstract

Aim of study: The aim of this study is to evaluate intraspecific variations of *Pinus pinea* needle essential oils obtained from five locations in Turkey

Area of study: The chemical composition of needle essential oil of *Pinus pinea* from five locations in Turkey was investigated and compared in order to determine differences among the regions (west; Armutlu and Kozak: east; Coruh, Maras Kapıcam and Onsen.

Material and methods: Each sample set containing fresh needles (700-800 g), were distillated with 3 L of water for 2 h by means of hydro distillation. Analyses were carried out in the GC-MS instrument to identify and quantitate the compounds in the EOs.

Main results: The results showed needle essential oil (EO) was dominated by monoterpene hydrocarbons (69.00- 84.90%), sesquiterpene hydrocarbons (4.10-8.80 %) and diterpenes (1.02-1.57%). As expected, the essential oil composed mainly of limonene (52.70-76.10%), β-phellandrene (3.19-7.20 %), α-terpineol (1.46-9.88 %) β-caryophellene (1.55-3.26 %) and germacrene D (0.67-2.43%). Similar to Italy and Algeria, the EOs from Turkey had limonene content greater than fifty percent, this percentage was lower in EOs from Greece and Tunisia.

Highlights: Cluster analysis ensured the separation of three groups where the first group was the eastern stands Coruh and Kahramanmaras Kapıcam with higher amount of limonene and lower amount of β-caryophyllene. The other group western stands Armutlu and Kozak were exhibiting lower amounts of limonene and a higher amount of α-terpineol.

Keywords: *Pinus pinea*, GC-MS Analysis, Essential Oil, Terpene, Hierarchical Cluster Analysis

Türkiye'de Fıstık çamı (*Pinus pinea*) İbresi Uçucu Yağının Coğrafi Varyasyonu

Öz

Çalışmanın amacı: Türkiye'de beş lokasyondan elde edilen *Pinus pinea* ibre uçucu yağlarının kimyasal bileşimi tür içi varyasyonlarını belirlemektir.

Çalışma alanı: Bölgeler arası farklılıkları belirlemek için Türkiye'deki beş lokasyondan (Doğu-Çoruh, Kahramanmaraş-Kapıçam, Kahramanmaraş-Önsen, Batı-Armutlu ve Kozak) *Pinus pinea* taze ibre örnekleri toplanmıştır.

Materyal ve yöntem: Her bir numune grubu için taze ibre örnekleri (700-800 g) 3 litre suda 2 saat boyunca hidrodestilasyon yöntemiyle işlendi. Uçucu yağlardaki bileşikleri belirlemek ve ölçmek için GC-MS cihazında analizler yapıldı.

Temel sonuçlar: İbre uçucu yağında monoterpen hidrokarbonların (% 69.00-84.90), seskiterpenlerin (% 4.10-8.80) ve diterpenlerin (% 1.02-1.57) baskın olarak bulunduğu görüldü. Sonuçlardan incelendiğinde, uçucu yağ esas olarak limonen (%52.70-76.10), β-phellandrene (%3.19-7.20), α-terpineol (% 1.46-9.88), β-karyofillen (%1.55-3.26) ve germakren D'den (0.67-%2.43) oluşmaktadır. Türkiye'den elde edilen uçucu yağın İtalya ve Cezayir'e benzer şekilde %50'den fazla limonen içeriğine sahip olduğu bu oranın Yunanistan ve Tunus'tan elde edilenlerde daha düşüktür.

Araştırma vurguları: Kümeleme analizi ile üç gruba ayrılmasını sağlamıştır. Birinci grup doğu meşcereleri olan Çoruh ve Kahramanmaraş Kapıcam olmak üzere daha yüksek limonen ve daha düşük miktarda β-karyofillen içeren şeklinde tanımlanırken diğer grup ise batı bölgeleri Armutlu ve Kozak, daha düşük miktarlarda limonen ve daha yüksek miktarda α-terpineol ile tanımlanmıştır.

Anahtar Kelimeler: *Pinus pinea*, GC-MS Analizi, Uçucu Yağ, Terpen, Aşamalı Kümeleme Analizi

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Introduction

Pinus pinea is widely spread in the Mediterranean region; Europe, North Africa and Asia. One of the five pine native species in Turkey is *Pinus pinea* (stone pine) exhibits the widest distribution. 50 % of stone pine forest is located in West part of Turkey, İzmir-Bergama, Aydın and Mugla, and a small part in Kahramanmaraş, Artvin and Armutlu (Akkemik et al., 2010). Because this species has economically valuable seeds, its tree is protected by inhabitants. Therefore, the wood and resin from stone pine is not harvested in Turkey.

The essential oil (EO) of stone pine needles was investigated in many Mediterranean countries. *Pinus pinea* EO was analyzed by researchers from Greece (Roussis et al., 1995), Italy (Macchioni et al., 2003), Tunisia (Nasri et al., 2011; Amri et al., 2012), Jordan (Halub et al., 2019), and Algeria (Fekih et al., 2019). Several investigations revealed that the EO of *Pinus pinea* needles mainly contains limonene, α-pinene and β-phellandrene.

The chemical composition and antimicrobial properties of pinea EO from Turkey were investigated by Demirci et al. (2015) considering only one district.

The aim of this study is to evaluate intraspecific variations of *Pinus pinea* needle essential oils obtained from five locations in Turkey (Coruh, Kahramanmaras-Kapicam and Onsen, Kozak, Armutlu). Additionally, the cluster analyses are applied by using volatile compositions to sample location from discriminate compounds.

Materials and Methods

The fresh needles of *Pinus pinea* were collected from five locations; Coruh (8 samples, 385 meters above sea level), Maras (Kapıcam, 16 samples, 610 m a.s.l), Maras (Onsen 8 samples, 705 m a.s.l), Kozak (6 samples, 470 m a.s.l) and Armutlu (6 samples, 330 m a.s.l) (Figure 1).

Each sample set containing fresh needles (700-800 g), were distilled with 3 L of water for 2 h by means of hydro distillation. The volatile compounds of the essential oil were extracted with petroleum ether (bp 40-60). The essential oil was stored at 0^{o}C maximal 2-3 days until analyzed. Analyses were carried out in the GC-MS (Shimadzu, QP 5050A) instrument to identify and quantitate the compounds in the EOs. Identification of most compounds was based on the libraries NIST 21, NIST 107 and WILEY 229. A private MS-data (Ucar library) library and Adam's library (2007) were also used to identify some compounds. Analyses were carried out on 30 m nonpolar fused silica DB-1 and DB-5 columns (0.25 mm, 0.25 µm film thickness), with a helium flow rate of 1.0 mL/min and a split ratio of 10:1. The following temperature program was maintained: 5 min at 60 ^{o}C, 3 ^{o}C /min to 120 ^{o}C, 5 ^{o}C /min to 200 ^{o}C, 10 ^{o}C /min to 260 ^{o}C and 8 min at 260 ^{o}C. Compound identification was also verified by comparing of their RI (Kovats indices) relative to C5–C24 n-alkanes obtained on a nonpolar DB-5MS column, with those provided in the literature Adams (2007) and Wiley library. Statistical analysis: a cluster analysis was conducted on the distribution of data by means of commercial SPSS software version 14.0 for Windows.

Results and Discussions

The composition of *Pinus pinea* fresh needle essential oils from five different locations is presented in Table 1. Ninety-six compounds identified; makeup about 97.60 to 98.90 % of the total needle essential oils. The EOs were dominated by monoterpene hydrocarbons (69.00- 84.90%), sesquiterpene hydrocarbons (4.10-8.80 %) and a slightly higher content of diterpenes (1.02-1.57%).

GC-MS analyses revealed that the major constituents of essential oils were limonene (52.70-76.10%), β-phellandrene (3.19-7.20 %), α-terpineol (1.46-9.88 %) β-caryophyllene (1.55-3.26 %) and germacrene D (0.67-2.43%). Despite a similar qualitative profile, prominent quantitative differences were observed among the needle essential oils of five locations. Among diterpene components, 11,13-labdadien-8-ol was detected with a significant amount in all essential oils. In comparison to the EOs, limonene content was higher in the eastern region of Turkey, especially Coruh which is the gene forest of *Pinus pinea*. The EO from Kahramanmaras Onsen differs from other regions because of its higher amount linalolol, β-ocimene and α-terpineol. The western part EOs (Kozak and Armutlu) were found to contain a slightly higher amount of germacrene D and guaiol. A similar pattern of major terpenes has already been reported for needle essential oils of *P. pinea* from different countries. The main compounds were found to be limonene (39.05%), β-phellandrene (13.80%), α-pinene (5.13%) in Greece (Roussis et al., 1995), limonene (54.60%), β-phellandrene (7.40%), α-pinene (4.00%), myrcene (2.40%) and α-phellandrene (2.40 %) in Turkey (Demirci et al., 2015).

Figure 1. Sampling sites of the fresh needles of *Pinus pinea*

Table 1. Essential oil composition of *Pinus pinea* needles

No.	Compound	RI	Coruh Mean %	Kapıcam Mean %	Onsen Mean %	Kozak Mean %	Armutlu Mean %
1	**α-Pinene**	938	**1.14**	**1.51**	**1.20**	**1.85**	**2.27**
2	Camphene	951	0.02	0.03	0.02	0.03	0.04
3	Sabinene	972	0.02	0.01	n.d.	0.02	0.05
4	**β-Pinene**	975	**0.38**	**0.69**	**0.40**	**0.84**	**1.01**
5	Dehydroxy linalool oxide	987	0.17	0.13	0.14	0.12	0.20
6	**Myrcene**	989	**1.34**	**1.53**	**1.55**	**1.55**	**1.74**
7	Dehydroxy linalool oxide <iso->	999	0.14	0.13	0.13	0.10	0.13
8	α-Phellandrene	999	0.19	0.23	0.17	0.49	0.42
9	α-Terpinene	1013	0.08	0.12	0.10	0.22	0.17
10	p-Cymene	1016	0.12	0.12	0.13	0.13	0.20
11	**β--Phellandrene** (w. 0.05-0.1% 1.8-Cineol)	1024	**3.99**	**5.78**	**3.19**	**7.20**	**3.52**
12	**Limonene**	1027	**76.14**	**73.92**	**52.74**	**55.29**	**62.13**
13	Cis-β-ocimene	1035	0.02	0.04	0.16	0.05	0.05
14	Trans-β-ocimene	1046	0.17	1.00	**5.00**	1.19	1.39
15	γ-Terpinene	1055	0.09	0.12	0.13	0.16	0.16
16	Linalool oxide <cis-> (furanoid)	1064	0.27	0.10	0.35	0.15	0.14
17	α-Terpinolene	1082	0.35	0.35	0.48	0.59	0.43
18	**Linalool**	1090	0.25	0.14	**3.85**	0.17	0.23
19	p-Menth-2-en-1-ol <cis->	1107	0.04	0.06	0.08	0.14	0.12
20	p-Menth-2-en-1-ol <tr->	1125	0.05	0.05	0.03	0.09	0.06
21	β-Terpineol <cis->	1129	0.14	0.11	0.21	0.19	0.14
22	β-Terpineol <trans->	1147	0.05	0.04	0.05	0.06	0.03
23	α-Phellandren-8-ol <cis->	1149	0.02	0.02	0.08	0.06	0.04
24	Borneol	1150	0.03	0.02	0.06	0.06	0.06
25	4-Terpineol	1163	0.23	0.23	0.46	0.41	0.30
26	a-Phellandren-8-ol <trans->	1171	0.02	0.01	0.06	0.02	0.03
27	**α–Terpineol**	1175	**1.62**	**1.46**	**9.88**	**3.43**	**1.85**
28	Neodihydrocarveol	1178	0.09	0.09	0.13	0.10	0.12
29	γ-Terpineol	1180	0.14	0.12	0.23	0.21	0.16
30	1-p-Menthen-9-al <cis->	1190	0.35	0.15	0.15	0.27	0.19
31	1-p-Menthen-9-al <trans->	1192	0.33	0.13	0.13	0.24	0.17
32	Carveol <trans->	1197	0.03	n.d.	0.03	0.10	0.13
33	Dihydrocarveol <iso->	1199	n.d.	n.d.	0.06	0.03	0.05

Table 1. Continued

			Coruh	Kapıcam	Onsen	Kozak	Armutlu
			Mean	Mean	Mean	Mean	Mean
No.	Compound	RI	%	%	%	%	%
34	Carveol <cis-->	1210	0.02	0.01	0.01	0.08	0.07
35	Carvone	1214	0.01	n.d.	n.d.	0.06	0.10
36	Thymol, Me-Ether	1219	0.16	0.02	n.d.	0.03	0.50
37	Piperitone	1226	0.03	0.07	0.09	0.18	0.14
38	Carvacrol, Me-Ether	1228	0.55	0.60	0.33	0.92	0.50
39	Geraniol	1240	n.d.	n.d.	0.15	n.d.	n.d.
40	2-Decenal	1242	0.04	n.d.	0.06	0.08	0.05
41	Bornyl acetate	1270	0.08	0.13	0.20	0.21	0.21
42	tr-Linolool oxid acetate (pyranoid)	1272	0.03	n.d.	0.08	n.d.	n.d.
43	Eugenol	1328	0.01	n.d.	n.d.	0.12	0.03
44	δ-Elemene <iso->	1331	0.01	n.d.	0.05	0.03	0.02
45	δ-Elemene	1333	0.09	0.15	0.21	0.32	0.23
46	Citronellyl acetate	1341	0.01	n.d.	0.11	0.05	0.03
47	α-Longipinene	1346	0.22	0.23	0.29	0.21	0.24
48	Neryl acetate	1364	0.01	n.d.	0.01	0.11	0.07
49	α-Ylangene	1367	0.04	0.03	0.05	0.04	0.04
50	α-Copaene	1371	0.03	0.02	0.06	0.02	0.04
51	Me-Eugenol	1376	0.08	0.07	n.d.	0.36	0.19
52	β–Bourbonene	1378	0.04	0.01	0.01	0.05	0.08
53	β-Elemene	1384	0.05	0.05	0.05	0.07	0.07
54	Longifolene	1394	0.55	0.52	0.63	0.39	0.63
55	**β-Caryophyllene <E>**	1409	**1.55**	**1.96**	**2.67**	**2.66**	**3.26**
56	Geranyl acetone	1431	0.07	0.08	0.05	0.06	0.07
57	α-Guaiene	1432	0.04	0.05	0.14	0.12	0.14
58	Aristolene (Guiadiene 6,9)	1434	0.22	0.27	0.32	0.51	0.54
59	Aromadendrene	1439	0.10	0.11	0.16	0.23	0.23
60	α-Humulene	1444	0.34	0.44	0.59	0.57	0.66
61	β-Farnesene <E>	1450	n.d.	n.d.	0.12	0.12	0.12
62	n-Dodecanol	1462	0.08	0.02	0.05	0.21	0.07
63	Phenyl ethyl isovalerate	1463	0.07	0.04	0.13	0.02	0.04
64	Phenyl ethyl valerate	1467	0.25	0.12	0.44	0.10	0.15
65	γ-Muurolene	1468	0.05	0.05	0.09	0.22	0.19
66	**Germacrene D**	1471	**0.85**	**0.67**	**0.67**	**2.43**	**1.88**
67	β-Selinene	1476	n.d.	n.d.	0.03	0.05	0.05
68	δ-Selinene	1481	0.25	0.35	0.48	0.84	0.82
69	α-Muurolene	1490	0.04	0.02	0.07	0.16	0.13
70	δ-Amorphene	1496	0.08	0.10	0.12	0.18	0.19
71	γ-Cadinene	1501	0.03	0.04	0.06	0.12	0.12
72	δ-Cadinene	1512	0.08	0.09	0.14	0.32	0.28
73	α-Bisabolene & Elemol	1534	0.04	0.04	0.19	0.11	0.11
74	Caryophyllene oxide	1566	0.05	0.04	0.07	0.10	0.18
75	β-Guaienol	1577	0.13	0.14	0.23	0.34	0.31
76	**Guaiol**	1583	**0.79**	**0.83**	**1.32**	**2.18**	**1.86**
77	Rosifoliol	1591	0.04	0.02	0.09	0.15	0.16
78	Selina-6-en-4-ol	1599	0.19	0.20	0.40	0.45	0.39
79	γ-Eudesmol	1615	0.08	0.09	0.21	0.30	0.19
80	β-Eudesmol	1632	0.07	0.06	n.d.	0.17	0.15
81	α-Cadnol&Eudesmol iso	1635	0.08	0.08	0.08	0.11	0.11
82	α-Eudesmol	1637	0.14	0.15	0.13	0.25	0.15
83	γ-Eudesmol epi	1640	0.03	0.03	0.25	0.37	0.32
84	a-Bisabolol	1667	n.d.	n.d.	0.03	0.04	0.04
85	Farnesyl acetate (2Z, 6E)	1822	0.03	0.03	0.04	0.20	0.10
86	n-Hexadecanoic acid	1946	0.04	0.01	0.02	0.10	n.d.
87	Pimaradiene	1949	0.01	n.d.	0.03	0.01	0.06
88	Manool oxide	1990	0.04	0.02	0.04	0.10	0.07
89	Manool oxide <13-epi->	2014	n.d.	n.d.	0.02	0.07	0.11
90	Abiatadiene	2084	0.02	0.01	0.02	0.05	0.05
91	**11,13-Labddien-8-ol**	2134	**0.83**	**1.14**	**0.88**	**0.99**	**0.86**
92	Abietal	2223	0.06	0.03	0.04	0.06	0.05
93	Me-Palustrate&Me-Levopimarate	2293	0.20	0.01	0.27	0.28	0.17
94	Me-Dehydroabietate	2314	0.03	0.01	0.03	0.07	0.05
95	Me-Abietate	2364	0.02	0.01	0.03	0.14	0.06
96	Me-Neoabietate	2416	0.02	0.01	0.02	0.08	0.03
	Sum.		**98.48**	**98.86**	**97.95**	**98.15**	**97.63**
RI	RI m/z (rel. int.)						
n.d.	Not determined						

Two studies in Tunisia had quite similar compounds, limonene (35.90%) and α-pinene (6.40%) (Nasri et al., 2011) and limonene (54.10%) and α-pinene (7.65%) (Amri et al., 2012). However, perhaps the most distinct chemical profile was demonstrated for *P. pinea* by Halub et al. (2019), who detected mainly guaiol (12.70%), limonene (11.42%), and β-caryophyllene (7.61%) in Jordan. Another study revealed different main compounds in the needles of *P. pinea* essential oil were α-pinene (37.00%), and farnesyl acetate (9.00%) in Morocco (Hmamouchi et al., 2001). *P. Pinea* grown in Italy the EO from needles contained limonene (58.90%), β-phellandrene (6.70%), α-pinene (6.20%) and β-caryophyllene (3.90%) (Macchioni et al., 2003) whereas the EO from braches included a higher amount of limonene (75.30%), 1.8-cineole (4.00%) and β-caryophyllene (3.70%) (Macchioni et al., 2002).

The wood EO in Lebanon dominated by limonene (65.40%), β-caryophyllene (17.30%) and α-terpineol (5.00%) (Saab et al., 2012). Fekih et al. (2019) reported that limonene (56.50%), α-pinene (6.50%) and β-phellandrene were the main components in EO of pinea needle in Algeria.

These studies were reported a significant difference in the volatile composition of the essential oil which may be due to the geographic location, climatic conditions and time of collection. Limonene which is the characteristic compound of *Pinus pinea* EO was over 50% in our samples. While the EOs from Turkey, Italy and Algeria had greater than 50% limonene, this rate was lower in EOs from Greece and Tunisia. The differences among the essential oils of pinea needles are mostly referred to as limonene, α-terpineol, α-pinene and β-phellandrene.

Taking into account our research and the data reported in literature, there is a noticeable predominance of the same five volatiles, but in a different order of concentrations. The needle essential oils from Tunisia and Algeria contained limonene and α-pinene as the main compounds. The average chemical profile of the dominant metabolites (contents>1%) in the essential oils of the needle may be presented as follows:

Limonene >> α-pinene > β-caryophyllene
whereas limonene and β-phellandrene were dominant compounds in Turkey, Italy and Greece:
Limonene >> β-phellandrene > α-terpineol or α-pinene > β-caryophyllene = germacrene D

Cluster Analysis

Also, a cluster analysis was conducted on the distribution of 38 compounds of Table 1 using commercial SPSS software. As shown in Figure 2, the five locations of *P. pinea* divided into three groups according to this statistical evaluation. The eastern stands Çoruh and Maraş Kapıcam with higher amount of limonene and lower amount of β-caryphyllene formed the first group, while the second group of western stands Armutlu, Kozak shows lower amounts of limonene and higher amount of α-terpineol. The remaining stand Kahramanmaraş Onsen1 and 2 exhibiting lower percentages of limonene (< 50%) and higher percentages of guaiol and all samples from Onsen showed a higher amount of linalool and β-ocimene represented the other group.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: M.B.U.; O.G.; Investigation: M.B.U.; O.G.; Material and Methodology: M.B.U.; O.G.; Supervision: M.B.U.; O.G.; Visualization: M.B.U.; O.G.; Writing-Original Draft: M.B.U.; O.G.; Writing-review & Editing: M.B.U.; O.G.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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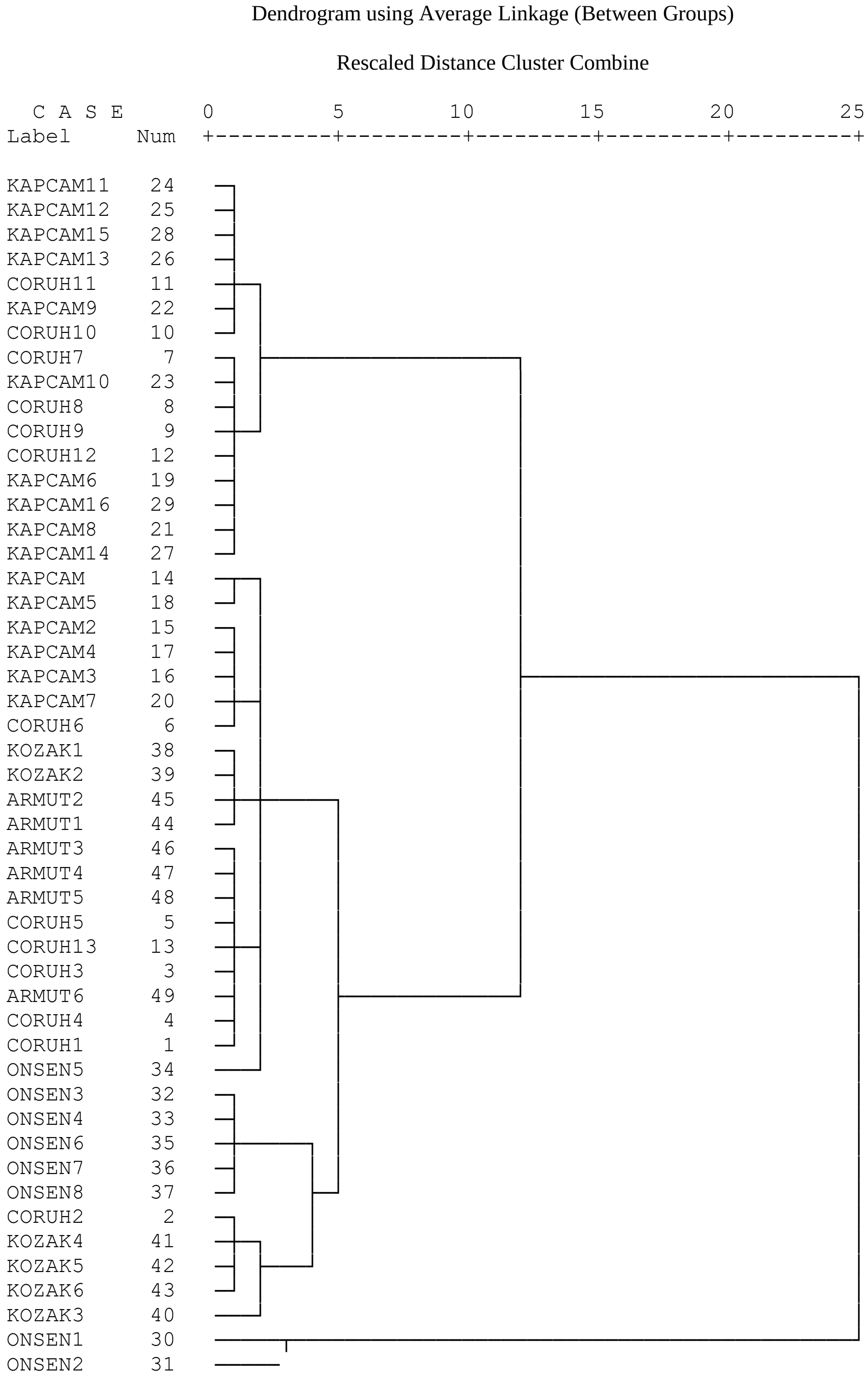

Figure 2. Hierarchical cluster analysis of the 38 compounds

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