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Chemical Polymorphism of Essential Oils from Populations of *Laurus nobilis* Grown on Tunisia, Algeria and France

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The compositions of the essential oils isolated from the aerial parts of tree Mediterranean populations of *Laurus nobilis* L. collected during the flowering phase on Tunisia, Algeria and France, were studied by GC and GC-MS. The analysis has allowed identifying 54 components. The main components were 1,8-cineole, α-terpinyl acetate (10-18.6%), methyl eugenol (10-22.1%), sabinene (1.2-8%), eugenol (1.2-11.7%) α-pinene (tr-4.5%) and β-pinene (0.4-4.2%). The monoterpene fraction was dominant in all the oils analysed and consisted mainly of oxygenated monoterpenes. The oils from the tree populations studied showed a clear chemical polymorphism. The principal component and the hierarchical cluster analyses separated the *Laurus nobilis* leaf essential oils into three groups.

Keywords: Essential oils, chemical polymorphism 1,8-cineole, *Laurus nobilis*, Mediterranean.

Lauraceae, one of the basal angiosperm families with fossils dating back to 100 million years ago [1], comprises 32 genera and about 2000-2500 species. The genus *Laurus* is considered as an ancient element of the Tertiary laurifolius flora [2] made of angiosperms (mostly Lauraceae) with large, thick evergreen leaves, which dominated the late Tertiary flora (Miocene and Pliocene, ca. 24 million years). This flora has now almost vanished from the Mediterranean and, except in the Azores, Madeira and the Canary Islands (Macaronesian islands), can only be found in restricted areas under humid and warm climates, although *L. nobilis* was initially described using material from Italy and Greece by LINNAEUS (1753) [3].

It is considered native only to Turkey (Anatolia) and the Balkan Peninsula [4]; Since antiquity, *Laurus nobilis* L., (bay) named Apollo's Laurel in mythology, was considered a plant native to the southern Mediterranean region and widely cultivated mainly in Europe and the USA as an ornamental plant [5]. It is considered as introduced elsewhere in the Mediterranean Basin [6]. Recently Bay is a plant of industrial importance, used in foods, drugs, and cosmetics. The dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups and fishes. Its antimicrobial and insecticidal activities are other factors for which bay are used in the food industry as a food preservative. The essential oil is also used as a folk medicine, especially for the treatment of rheumatism and dermatitis [7]. In this paper we analyzed the composition of essential oil for *Laurus nobilis* grown on Tunisia, Algeria and France, in the purpose to reveal the existence of different chemotypes either to reveal resemblances within the chemical composition of the essential oil of laurel on both sides of the Mediterranean.

The chromatographic analyses (GC and GC/MS) of the essential oils allowed the identification of 54 components (Table 1) representing 98.1 to 99.0% of the total oil content. The identified components were divided in five chemical classes (Table 2). The major class was constituted by the oxygenated monoterpenes (54.8 – 58.9%), with 1,8-cineole (21.9 - 32.9%) having the highest content in all samples analyzed for the three populations, followed by α-terpinyl acetate (11.9 - 14.5%), linalool (4.4 -13.8%), terpinen -4-ol (2.4 - 3.1%) and α-terpineol (1.7 - 2.8%). The class with the second highest contents was composed of the phenylpropanoids (16 - 24.4%), represented by methyl eugenol (13.2 - 16.6%), eugenol (2.0 - 6.4%) and elemicin (0.7 - 1.8%). The monoterpene hydrocarbons (9.7 - 17.5%) were composed essentially of α-pinene (2.4 - 4.2%), sabinene (3.6-5.6%), and β-pinene (1.9 - 3.0%). The principal compound of oxygenated sesquiterpenes (fourth compound class with a content reaching between 3.5 and 5.9%) was spathulenol (1.1-2.1%) and caryophyllene oxide (0.7-1.9%). The sesquiterpene hydrocarbons, were minor

compound classes (2.4 - 7.8%) with (*E*)-caryophyllene having the highest content (0.7-2.6%).

The essential oil of the different populations contained the same compounds, but the quantitative difference, between all main compounds were quite large. Tunisian leaf essential oil presented the highest mean percentage of 1,8-cineole, contained the highest percentage of monoterpene but was poor in compounds belonging to the other chemical classes. French samples were relatively rich in phenylpropanoids but presented the low percentage of monoterpene hydrocarbons. Algerian leaf essential oil was characterized by the highest mean percentage of sesquiterpenes.

The principal component analysis (PCA), performed on average contents of all constituents for each population, showed that the first two principal axes represent 98.3% of the total variation. The first axis (60.0% of the total variation) was mainly correlated with sabinene, 1,8-cineole, linalool, terpinen-4-ol, nerol, (*Z*)-β-ocimene, β-longipinene and δ-cadinene. The second axis represented 38.3% of the total variation, and α-terpinyl acetate, *p*-cymene, germacrene D, bicyclogermacrene and α-himachalene were the main variables contributing to its definition. The third principal component (only 1.2% of the variation) was correlated with eugenol and *p*-cymene.

The plot of the projection of the variables and sample values onto the first two principal components identified three significantly different chemical groups (Figure 1). The analysis revealed a high chemical structure among populations.

Tunisian Population is situated at the positive side of Axes 1 and 2. This population is characterized by oil rich in 1,8-cineole, α-pinene and sabinene then other two populations; While Algerian and French populations are situated at the negative side of the vertical axis (Axis 1). Algerian oil contain linalool in the biggest quantity, French oil content the highest percent of α-terpinyl acetate, eugenol and methyl eugenol similar results are reported by Marzouki et al[8] when they reveled important quantitative difference in the chemical composition of oil from Tunisia and Algeria. The result is so in accordance with other earlier studies on *L. nobilis* from Iran [9], the main components of the oil were identified. 1,8-Cineole was the major component in the oil together with β-terpinyl acetate, terpinene-4-ol, α-pinene, β-pinene, *p*-cymene, linalool and terpinene-4-yl acetate.

The dendrogram generated from the Euclidean distances among pairs of populations showed population groupings globally similar to those observed by the PCA, Three populations are clearly separated. The UPGMA dendrogram identified two significantly different groups, one including samples from France and Algeria, and the other including Tunisian population (Figure 2).

Table 1: Retention Indices (RI) and percent average contents of constituents of the essential oil for every population of *Laurus nobilis* L. (%W/W).

No	RI	Compound	France	Algeria	Tunisia
1	926	Tricyclene [a,b]	0.23	0.40	0.53
2	934	α-Pinene[a,b,c]	2.39	3.12	4.20
3	948	Camphene [a,b]	0.25	0.08	0.65
4	974	Sabinene[a,b,c]	3.65	4.10	5.60
5	978	β-Pinene[a,b,c]	1.91	2.32	3.07
6	992	Myrcene[a,b,c]	0.18	0.10	0.43
7	1006	α-Phellandrene[a,b,c]	Tr	Tr	Tr
8	1012	Δ^2-Carene [a,b]	0.12	0.38	0.54
9	1017	Δ^3-Carene [a,b]	0.09	0.28	0.42
10	1022	*o*-Cymene [a,b]	Tr	0.08	0.11
11	1024	*p*-Cymene [a,b]	0.41	0.06	0.39
12	1036	1,8-Cineole[a,b,c]	21.87	23.02	32.90
13	1055	*Z*-β-Ocimene [a,b]	Tr	Tr	Tr
14	1058	γ-Terpinene[a,b,c]	0.45	0.46	0.95
15	1066	*cis*-Sabinene hydrate [a,b]	0.03	0.16	0.32
16	1089	Terpinolene[a,b,c]	0.03	0.18	0.31
17	1102	Linalool[a,b,c]	12.64	13.82	4.45
18	1138	*trans*-Pinocaveol [a,b]	0.02	0.02	0.04
19	1165	Borneol[a,b,c]	0.17	0.04	0.57
20	1167	*p*-Mentha-1,5-dien-8-ol[a,b]	0.09	0.32	0.35
21	1178	Terpinen-4-ol[a,b,c]	2.51	2.40	3.12
22	1191	α-Terpineol [a,b]	1.71	2.54	2.82
23	1215	*cis*-Sabinene hydrate acetate [a,b]	Tr	Tr	Tr
24	1228	Nerol [a,b]	0.04	0.10	0.22
25	1234	Linalool acetate [a,b]	0.75	0.24	0.08
26	1286	Bornyl acetate[a,b,c]	0.24	0.04	0.41
27	1318	Iso-3-thujyl acetate [a,b]	0.71	0.32	0.63
28	1354	α-Terpinyl acetate[a,b,c]	14.56	11.98	13.37
29	1361	Eugenol[a,b,c]	6.42	2.78	2.04
30	1366	α-Yalangene [a,b]	Tr	0.28	0.02
31	1390	β-Cubebene [a,b]	Tr	Tr	Tr
32	1392	β-Longipinene [a,b]	0.85	0.84	0.51
33	1410	Methyl eugenol[a,b,c]	16.58	14.02	13.22
34	1416	α-Gurjunene [a,b]	Tr	0.08	Tr
35	1419	*E*-Caryophyllene[a,b,c]	1.06	2.68	0.7
36	1438	α-Guaiene [a,b]	0.15	0.65	0.02
37	1445	*cis*-Muurola-3,5-diene [a,b]	Tr	Tr	Tr
38	1453	α-Himachalene [a,b]	Tr	0.34	Tr
39	1480	Germacrene D [a,b]	Tr	0.52	0.21
40	1494	*cis*-β-Guaiene [a,b]	0.29	0.72	0.11
41	1496	Bicyclogermacrene [a,b]	0.26	1.00	0.39
42	1501	Viridiflorene [a,b]	Tr	0.02	Tr
43	1505	α-Bulnesene [a,b]	Tr	0.08	0.07
44	1513	*trans*-Cadinene [a,b]	0.24	0.04	0.06
45	1524	δ-Cadinene [a,b]	0.62	0.62	0.38
46	1559	Elemicin [a,b]	1.44	1.84	0.74
47	1577	Spathulenol [a,b]	1.79	2.1	1.15
48	1582	Caryophylene oxide[a,b,c]	1.38	1.98	0.76
49	1590	Globulol[a,b]	0.38	0.18	0.16
50	1645	1-Epicubinol [a,b]	0.39	0.2	0.33
51	1648	β-Eudesmol [a,b]	0.13	0.2	0.24
52	1654	α-Cadinol [a,b]	0.84	1.02	0.58
53	1668	*Z*-Nerolidol acetate [a,b]	0.08	0.02	0.02
54	1673	5-Izocedranol [a,b]	0.19	0.22	0.26
Monoterpene hydrocarbons			9.74	11.7	17.6
Oxygen containing monoterpenes			55.31	54.8	59
Sesquiterpene hydrocarbons			3.47	7.87	2.47
Oxygen containing sesquiterpenes			5.18	5.92	3.5
Phenylpropanoids			24.44	18.6	16
Total identified			98.14	99	98.5

Identification has been realized by comparing mass spectra (a), retention indices (b), and injection of authentic compound (c).

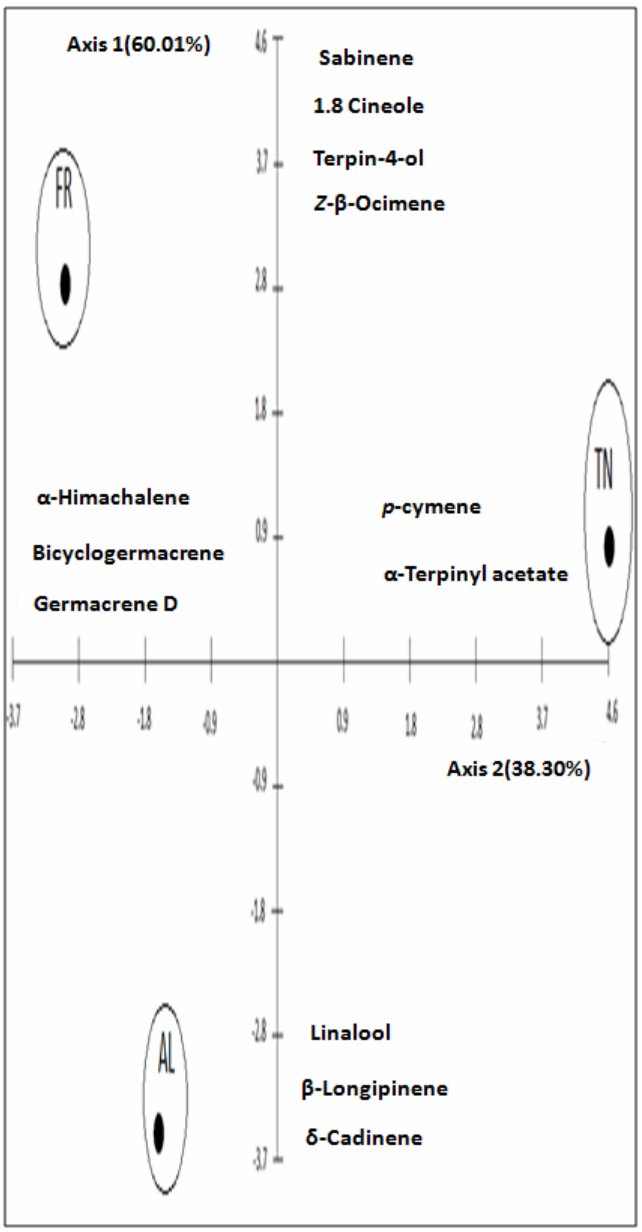

Figure 1: Principal Component Analysis for the leaf essential oil constituents of the 3 *Laurus nobilis* L. populations. Projection of the contents of the oil constituents onto the first two principal axis († and – indicate positive and negative correlations with the axes, resp.). Ellipses were drawn arbitrarily.

This high variability has been explained by the out crossing mating system and the floral biology [10,11] as well as by ecological (i.e., climatic and edaphic factors) [12] and genetic factors [13,14] . This study indicates that Medeterranean *L. nobilis* is 1,8-cineole chemical specie. The result of this research is in accordance with other earlier studies on *L. nobilis* that all found to be rich in 1,8-cineole [7,15,16]. The analysis of essentials oils showed important quantitative differences among Tunisian, Algerian and French populations. The essential oil from *L. nobilis* showed a clear chemical polymorphism, that was particularly evident among three populations growing in different locations

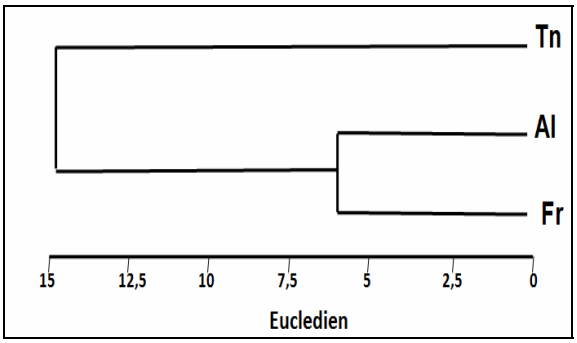

Figure 2: Dendrogram, produced with UPGAMA method, showing the Euclidean distances among the three populations of *Laurus nobilis*.L. The dendrogram was based on the essential oil data matrix

Experimental

Plant Material: Leaves were sampled from 5–10 *Laurus nobilis* L. trees in each of three populations, including Tunisian, Algerian and French population. In all populations leaves were collected in April 2007, in flowering period. At each site we collected from a single tree about 2 kg of leaves that were separated from the lignified parts and air dried in the shadow for 15 days. Voucher specimens of each sample were deposited in the herbarium of the Faculty of Pharmacy in Monastir (Tunisia). Codes of the samples are: Tn (1-10), Al (1-5) and Fr (1-10), respectively.

Oil Isolation: A 100 g amount of dried leaves boorishly crushed and mixed with 600 ml distilled water were subjected to HD for 4h using a modified Clevenger-type apparatus described by Simard *et al*. [17].

GC-MS Analysis: A gas chromatograph, GC, AGILENT Technologies Inc. (Santa Clara, CA, USA) model 6890N was employed for analysis of the extracts. It was equipped with a split-splitless injector, an autosampler AGILENT model 7683 and an AGILENT HP5 fused silica column; 5% phenylmethylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm. GC conditions used were: programmed heating from 60 to 280°C at 3°C/min followed by 30 min under isothermal conditions. Data were recorded after a 3 min lag phase. The injector was maintained at 250°C. Helium was the carrier gas at 1.0 mL/min; the sample (1 µL) was injected in the split mode (1:20). The GC was fitted with a quadrupole mass spectrometer, MS, AGILENT model 5973 detector. MS conditions were as follows: ionization energy 70 eV, electronic impact ion source temperature 200°C, quadrupole temperature 100°C, scan rate 1.6 scan/sec, mass range 50-500 u. Software adopted to handle mass spectra and chromatograms was ChemStation. NIST02 and LIBR (TP) [18-19] Mass Spectra Libraries were used as references. Samples, a mix of the oils obtained from three repeated hydrodistillations, were run in chloroform at a dilution ratio of 1:100. No repeated runs were performed.

Compound Identification: Compounds were identified by matching their mass spectra and retention times with those reported in the literature [18,19]. Moreover, identification of some compounds, among the more important constituents, has been confirmed by injection of authentic samples (see Table 1). The chromatographic results present in Table 1 are expressed as area percentages, calculated without any response factor, as a function of retention time.

Statistical Analysis: The chemical population structure and the relationship among populations were determined by principal component analysis (PCA) performed on the percentages of all identified constituents for all populations using the program Multi-Variante Statistical Package MVSP 3.1 [20]. The divergence between populations was also estimated by the Euclidean distances calculated among population pairs. The non-weighed pair-group method with arithmetic averaging (UPGMA) was used to construct a dendrogram representing all populations.

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