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Effect of geographical origin on yield and composition of cone essential oils of *Cedrus libani* A. Rich. growing in Lebanese protected areas and variability assessment in comparison with literature survey

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Abstract: Gas chromatography–mass spectrometry analysis together with principal component analysis revealed that geographical origin influenced the yield and composition of the essential oils (EOs) extracted by hydrodistillation performed for 3 h using a Clevenger-type apparatus, from the cones of *Cedrus libani* A. Rich., growing wild at four Lebanese natural reserves and protected areas: Bsharri, Chouf, Ehden, and Tannourine, and from a cultivated cedar growing in Qartaba. Essential oil chemical variability established between the different studied provenances suggested the involvement of abiotic factors such as geographical conditions, cultivation conditions, soil composition, and environmental factors in the chemical polymorphism of *C. libani* cones EOs. α-Pinene/β-pinene characterized Ehden (β-pinene 35.6%/α-pinene 27.7%), Chouf (α-pinene 37.3%/β-pinene 26.1%), Bsharri (α-pinene 27.7%/β-pinene 21.4%), and Tannourine (α-pinene 25.1%/β-pinene 16.0%) samples, whereas Qartaba EO was distinguished by the dominance of myrcene (30.6%), α-pinene(26%), and limonene (14.1%). Comparison with the existing literature reinforced the chemical variability of *C. libani* EOs. This current study helped the estimation of a best harvest location for a good EO quality production, resource optimization, and pharmacological properties evaluation, according to the market demand.

Keywords: *Cedrus libani*; chemical variability; essential oil; geographical location.

1 Introduction

Growing at a high altitude between 1200 and 2000 m, often in rocky and barely reachable locations, *Cedrus libani* A. Rich. is admired for its beauty and the imputrescibility of its wood [1]. *Cedrus libani* exists naturally in the Alaouite Mountains in Syria, in Toros Mountains in Turkey, and in the Coastal Mountains of Lebanon [2].

The cedar of Lebanon is mentioned 75 times in the Holy Bible [3]; owing to its majesty and its long life, this millennium tree represents the symbol of strength, stability, life, hope, freedom, continuity and eternity; hence, it is adopted as the national emblem of Lebanon [4].

Cedar of Lebanon is considered a tree of great historical and economic value providing a prized wood [5–7]. *Cedrus libani* essential oils (EOs) had a great usage in folk medicine. Since time immemorial, they were used to embalm and conserve the pharaohs of Egypt [3]. The existence of antimicrobial, antioxidant, larvicidal, and antiviral properties related to these oils has been proven in recent reports [2, 8–10].

Bearing in mind that a combination of more than one factor may affect the metabolomic profile of plants leading to specific EO chemical compositions and defining their biological properties [11–18], this present investigation aims to contribute to a better knowledge of the chemical composition of *C. libani* cones EOs and to follow the chemical variability according to region of harvest between cones of cedars growing wild at four Lebanese natural reserves and protected areas, Bsharri, Chouf, Ehden, and

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Tannourine, and cones of a cultivated cedar growing in Qartaba. Besides, the chemical diversity of the volatile components was described in comparison with the data available in the literature. In fact, EO chemical variability in response to biotic and abiotic pressures constitutes a significant correlation between chemical composition and bioactivity and procures a new source of information for the development of future optimal EO mixture.

To the best of our knowledge, studies investigating a detailed chemical characterization and polymorphism of Lebanese *C. libani* cones EOs, in relation to geographical location, have not been published to date. Furthermore, this is the first study occurring on different Lebanese natural reserves with a permission of harvest conferred by officials.

2 Experimental

2.1 Plant material and EO extraction

Cedrus libani cones were collected from cedars growing wild at four different Lebanese natural reserves and protected areas: Bsharri (1950 m) (Bsharri District/North Governorate), Ehden (1800 m) (Zgharta District/North Governorate), Tannourine (1850 m) (Batroun District/North Governorate), and Chouf (1650 m) (Chouf District/Mount Lebanon Governorate), and from a cedar cultivated at Qartaba (1200 m) (Jbeil District/Mount Lebanon Governorate).

The harvest was executed in September 2016, at 10 AM of each sampling date.

Botanical identification of the plant samples was carried out by Dr. Marc El Beyrouthy according to the New Flora of Lebanon and Syria as described by Mouterde in 1983 [19]. A voucher specimen of each cones sample was deposited in the Herbarium of the Faculty of Agricultural and Food Sciences of Holy Spirit University of Kaslik, Lebanon, under the registry numbers MNI015a for the Bsharri cedars, MNI015b for the Ehden cedars, MNI015c for the Tannourine cedars, MNI015d for the Chouf cedars, and MNI015e for Qartaba cedars. Three hundred grams of *C. libani* cones was air dried in the absence of light at 4 °C for a period of 4 weeks. The EOs were extracted by hydrodistillation performed for 3 h using a Clevenger-type apparatus, according to the method described in the European Pharmacopoeia, 1997 [20]. The extracted oils were kept, measured, and stored at 4 °C in tightly closed glass vials until their analysis by gas chromatography–mass spectrometry (GC/MS). Essential oil content, expressed in mL/g, was evaluated by measuring the volume of oil extracted per weight of dried plant material.

2.2 Essential oils analyses

2.2.1 GC analyses

The GC analysis was carried out using a Thermo Electron Corporation apparatus (Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector, a nonpolar HP-5MS (5% phenylmethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm), and a polar fused-silica HP Innowax capillary column (polyethylene glycol, 50 m × 0.20 mm i.d., film thickness 0.20 μm) in order to confirm the identification of the components. The carrier gas was He at a rate of 1 mL/min. The oven temperature followed a gradient rising from 40 °C to 300 °C at 5 °C/min and finally held isothermal at 300 °C for 5 min. Diluted 1-μL samples (1/100, vol/vol in pentane) were manually injected at 250 °C and in the splitless mode. Flame ionization detection was performed at 310 °C.

2.2.2 GC/MS analyses

The GC/MS analyses were achieved using an Agilent Thermo Electron Corporation (Agilent, Santa Clara, CA, USA) apparatus coupled with Mass Detector 5975 and equipped with 7683 B auto sampler [injection of 1 μL of each oil sample diluted in pentane (1/100 vol/vol)]. A fused-silica capillary column HP-5MS (30 m × 0.25 mm i.d., film thickness 0.25 μm) and a fused-silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm i.d., film thickness 0.20 μm) were used. The oven temperature program was identical to that described above (*cf.* GC analyses). Helium was the carrier gas (1 mL/min). The mass spectra were recorded at 70 eV. Ion source and transfer line temperatures were 310 °C and 320 °C, respectively. The acquisition was recorded in full scan mode (50–400 amu).

2.2.3 Identification and quantification of the components

An *n*-alkane (C_7-C_{25}) mixture was analyzed under the same experimental GC/MS conditions to calculate the retention indices (RIs). Identification of EO components was performed by comparing their mass spectra on both columns with those listed in the commercial mass spectral libraries NIST and Wiley 275 computer libraries, our home-made

library constructed with pure compounds, and those from literature [21, 22], allowing a reliable confirmation of the identity of each component. A further identification was accomplished by comparing their RIs on both polar and apolar columns relative to the retention times of the series of *n*-alkanes (C_7–C_{25}) with those from literature [21, 23] or with those of standard compounds available in our laboratories, obtained from Sigma-Aldrich (Germany). Standards of some EOs of known composition (such as the EO of *Rosmarinus officinalis* L. from Phytosun Aroms, Plélo, France) were also injected in similar conditions for the comparison of retention times (t_R) and mass spectra. In addition, the following standards were purchased from Sigma-Aldrich: α-pinene, camphene, β-pinene, myrcene, limonene, borneol, terpinen-4-ol, α-terpineol, verbenone, bornyl acetate, β-caryophyllene, γ-cadinene, δ-cadinene, caryophyllene oxide, α-cadinol, and β-bisabolol. Relative proportion of each compound was determined from the GC peak areas.

2.3 Statistical analyses

2.3.1 Analysis of variance

One-way analysis of variance (ANOVA) was applied in order to evaluate EO chemical variability according to geographical location and to identify where any difference may have existed between samples. Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). A probability value of $p \leq 0.05$ was considered to highlight a statistically significant difference between oil samples.

2.3.2 Principal components analysis

Principal component analysis (PCA), a multivariate statistical technique that aims at reducing the multivariate space in which objects (oil samples) are distributed, was applied. This processing was performed using XLSTAT 2014.5.03 (Addinsoft, Paris, France).

3 Results and discussion

3.1 Essential oils yield

The average of EO yields from the cones of cedars harvested in triplicate from each site of different Lebanese geographical locations fluctuated between 0.16% ± 0.02% and 0.54% ± 0.1%.

Cones of Chouf natural reserve presented the highest EO yield (0.54%), followed by those harvested from Ehden (0.29%), Bsharri (0.25%), and Tannourine (0.24%), whereas cones of the cultivated cedar from Qartaba exhibited the lowest EO yield (0.16%). It can be concluded that EO yield varies according to the provenance.

According to Tumen et al. [24], oil yield was 0.37% in “”*C. libani* cones from Adana, Turkey. Yilmaz et al. in 2005 reported that the yield obtained from *C. libani* cones harvested from Başkonuş District, Turkey, was 1% [25]. Loizzo et al. [8] in 2007 revealed that EO yield from cones of *C. libani* harvested from Hadath Eljebeh–Tannourine reserve was 0.41%.

The difference in yield observed in our results between the different sites asserts EO chemical variability, which is favored by the existence of a wide variety of microclimatic zones distinguishing Lebanon. In fact, chemical variability does not only depend on genetic background, but it can be also affected by external conditions or ecological factors that can have effects on plant development and vegetative growth and have a direct impact on EO production, both quantitatively and qualitatively. Those extrinsic factors include oil composition, cultivation conditions, and geographical factors such as altitude and climatic factors (temperature, sunshine duration, wind regime, rainfall, humidity, etc.) [16, 26].

3.2 Essential oils chemical characterization and polymorphism

3.2.1 Essential oil chemical composition

The EOs extracted from the cones of *C. libani* constitute a mixture of 51 compounds, representing 95.2% to 99.9% of the total oil composition. Table 1 lists the average of the relative percentages of the constituents of the oils, collected in triplicate from each site of the five Lebanese locations during September 2016. *Cedrus libani* EO chemical compositions were distinguished by their wealth in α-pinene (25.1%–37.3%), β-pinene (6.4%–35.6%), myrcene (0%–30.6%), limonene (5.6%–14.1%), sclarene (1.1%–5.5%), abieta-8,11,13-triene (2.2%–10.2%), kaur-16-ene (3.5%–7.5%), and abieta-7,13-diene (1.9%–7.5%), representing more than 5% of the total oil content in at least a sample, with a predominance of α-pinene, β-pinene, and myrcene (Table 1).

Table 1: Chemical composition of the EOs extracted from the cones of *C. libani* harvested from different Lebanese geographical locations.

RI[a]	RI[b]	Region of harvest	Bsharri	Ehden	Tannourine	Chouf	Qartaba
		Date of harvest	Sep-16				
		Essential oil yield (%)	0.25 ± 0.02	0.29 ± 0.04	0.24 ± 0.01	0.54 ± 0.01	0.16 ± 0.02
		Chemical compound					
933	1076	α-Pinene	27.7 ± 0.4	27.7 ± 0.3	25.1 ± 0.8	37.3 ± 0.4	26.0 ± 1.8
947	1076	Camphene	1.0 ± 0.0	1.3 ± 0.3	0.9 ± 0.1	1.8 ± 0.0	1.1 ± 0.1
980	1118	β-Pinene	21.4 ± 0.4	35.6 ± 1.8	16.0 ± 0.5	26.1 ± 0.1	6.4 ± 0.4
991	1174	Myrcene	ND	ND	ND	ND	30.6 ± 0.7
1009	1157	δ-3-Carene	0.5 ± 0.3	0.4 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.0
1025	1203	Limonene	9.8 ± 0.1	7.5 ± 0.2	5.9 ± 0.2	5.6 ± 0.2	14.1 ± 0.1
1057	1255	γ-Terpinene	ND	0.2 ± 0.0	ND	0.2 ± 0.0	ND
1086	1265	α-Terpinolene	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
1089	1282	*p*-Cymenene	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	ND
1094	1392	2-Nonanone	0.1 ± 0.0	0.1 ± 0.0	ND	0.1 ± 0.0	0.1 ± 0.0
1096	1401	Fenchone	ND	0.1 ± 0.0	ND	0.1 ± 0.0	ND
1117	–	*trans*-*p*-Menth-2-en-1-ol	ND	0.1 ± 0.0	ND	ND	ND
1128	1487	α-Campholenal	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	ND
1138	1664	*trans*-Pinocarveol	0.5 ± 0.0	0.9 ± 0.1	1.7 ± 0.1	0.7 ± 0.1	0.1 ± 0.0
1140	1663	*cis*-Verbenol	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.2	0.3 ± 0.1	0.2 ± 0.0
1145	1532	Camphor	ND	0.1 ± 0.0	ND	ND	0.0 ± 0.0
1159	1535	*trans*-Pinocamphone	ND	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	ND
1165	1587	Pinocarvone	ND	0.1 ± 0.0	ND	0.2 ± 0.0	ND
1167	1719	Borneol	0.1 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
1174	1611	Terpinen-4-ol	0.5 ± 0.1	1.0 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.1 ± 0.0
1185	1864	*p*-Cymen-8-ol	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
1201	1706	α-Terpineol	1.8 ± 0.1	2.01 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	0.2 ± 0.0
1205	1731	Verbenone	0.3 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.1 ± 0.0
1217	1845	*trans*-Carveol	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	ND
1238	1607	Thymol methyl ether	0.1 ± 0.0	ND	0.3 ± 0.0	0.1 ± 0.0	ND
1241	1752	Carvone	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
1284	1597	Bornyl acetate	2.3 ± 0.2	0.9 ± 0.1	0.5 ± 0.1	1.3 ± 0.6	0.6 ± 0.1
1300	1661	*trans*-Pinocarvyl acetate	0.2 ± 0.0	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	ND
1328	1698	Myrtenyl acetate	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	ND	ND
1378	1497	α-Copaene	ND	ND	ND	0.6 ± 0.2	ND
1379	1765	Geranyl acetate	0.5 ± 0.1	0.2 ± 0.0	ND	0.1 ± 0.0	ND
1416	1612	β-Caryophyllene	0.1 ± 0.0	ND	0.2 ± 0.1	0.1 ± 0.0	1.0 ± 0.1
1457	1689	β-Farnesene	1.9 ± 0.1	0.5 ± 0.0	4.0 ± 0.0	0.3 ± 0.0	01.0 ± 0.1
1500	1740	α-Muurolene	ND	ND	ND	0.1 ± 0.0	0.1 ± 0.0
1513	1776	γ-Cadinene	0.1 ± 0.0	0.2 ± 0.0	ND	0.1 ± 0.0	ND
1526	1773	δ-Cadinene	ND	0.2 ± 0.0	ND	ND	ND
1581	2008	Caryophyllene oxide	ND	ND	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
1653	2256	α-Cadinol	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	ND	ND
1668	2020	β-Bisabolol	0.2 ± 0.1	ND	ND	0.1 ± 0.0	ND
1724	1946	Farnesol	ND	ND	ND	ND	0.2 ± 0.1
1948	–	Cembrene	ND	ND	ND	ND	0.1 ± 0.1
1974	2408	Sclarene	4.7 ± 0.0	1.1 ± 0.1	5.5 ± 0.2	4.0 ± 0.1	2.6 ± 0.1
2057	2525	Abieta-8,11,13-triene	7.0 ± 0.2	7.7 ± 0.3	10.2 ± 0.7	3.0 ± 0.2	2.2 ± 0.5
2060	2438	Kaur-16-ene	7.1 ± 0.1	4.7 ± 0.5	7.5 ± 0.1	7.1 ± 0.1	3.5 ± 0.1
2098	–	Abieta-7,13-diene	3.4 ± 0.0	1.9 ± 0.1	7.5 ± 0.3	3.2 ± 0.1	4.7 ± 0.8
2268	–	Dehydroabietal	0.9 ± 0.0	0.9 ± 0.0	1.7 ± 0.0	0.5 ± 0.0	0.8 ± 0.6
2274	–	β-Cembrenediol	1.5 ± 0.0	1.2 ± 0.4	4.2 ± 0.2	0.4 ± 0.1	2.6 ± 0.1
		Monoterpene hydrocarbons	60.7	73.1	48.9	71.7	79.0
		Oxygenated monoterpenes	7.4	7.9	9.7	8.2	1.7
		Sesquiterpene hydrocarbons	2.1	0.9	4.2	1.3	2.1
		Oxygenated sesquiterpenes	0.3	0.2	0.4	0.3	0.4
		Others	24.7	17.7	36.6	18.4	16.6
		Total	95.2	99.8	99.8	99.9	99.8

The chemical compounds are presented as the average of the relative percentages of EOs. [a]RIs calculated on an HP-5MS column. [b]RIs calculated on an HP Innowax column. ND, Not detected.

3.2.2 Essential oil chemical variability according to the geographical origin

For the purpose of detecting a potential impact of harvesting sites on the accumulation of the main terpene metabolites in EO of *C. libani* from cones, which may lead to specific oil chemical compositions, one-way ANOVA test (SPSS 16.0 software) was applied in order to evaluate the existence of significant correlations between EO main components of cedar cones and the geographical location of harvest and to estimate the optimal harvesting site of *C. libani*, providing the optimal EO mixture corresponding to a required activity according to user's need.

One-way ANOVA test showed that EO chemical composition varied considerably according to the geographical location of harvest ($p < 0.05$).

Our study revealed the dominance of α-pinene in all the samples collected from the different Lebanese sites. In Chouf reserve, we noted the highest α-pinene contents.

β-Pinene amounts significantly fluctuated among the regions and were higher in the samples collected from Ehden reserve, while the lowest values of β-pinene were noticed in Qartaba ($p < 0.05$).

Limonene levels were significantly lower in Tannourine and Chouf, while this compound predominated in the EO of the cones harvested from Qartaba ($p < 0.05$).

The region of harvest largely affected myrcene ($p < 0.05$). A statistically significant difference in α-terpineol contents was noted between the regions ($p < 0.05$); the highest values were noticed in Tannourine and Chouf, whereas we registered the lowest levels in Qartaba.

Bornyl acetate amounts significantly varied between the locations ($p < 0.05$) and prevailed in the samples harvested from Bsharri and Chouf compared to the other sites.

Geographical location significantly affected sclarene, kaur-16-ene, abieta-8,11,13-triene, and abieta-7,13-diene levels ($p < 0.05$). Sclarene and kaur-16-ene reached their minimum values in Ehden reserve and Qartaba. The samples collected from Chouf reserve and Qartaba were characterized by their lowest abieta-8,11,13-triene amounts, whereas the samples harvested from Tannourine were distinguished by their wealth in this component. The lowest values of abieta-7,13-diene were recorded in Ehden and the highest ones in Tannourine.

Geographical locations influenced the contents of β-farnesene and β-cembrenediol ($p < 0.05$). Their amounts were significantly higher in Tannourine compared to the other provenances. The lowest values of β-farnesene were observed in Ehden and Chouf reserves and in Chouf reserve for β-cembrenediol.

3.2.3 Chemical variability of *C. libani* cones EOs according to geographical origin – comparison with the literature

In order to assess for the first time the existence of cedar cones EO chemical polymorphism within a group of samples collected from different geographical locations and to distinguish which constituent has signed the oil chemical identity in cedar cones EOs according to the provenance, PCA was conducted on all EO samples (Figure 1). Principal component analysis showed that the maximal amount of variance in the data and its direction are explained by the first two principal components PC1 and PC2, which extracted 72.01% and 10.92% of the total variance, respectively.

The first PC is mainly associated to α-pinene witnessing the predominance of this compound in all the oil studied samples and marking its lowest values in Adana, Turkey (8) [24]. β-Pinene, limonene, abieta-8,11,13-triene and abieta-7,13-diene contributed to the definition of the first PC.

Significant concentrations of β-pinene distinguished predominantly Ehden (2) (our study), Chouf (4) (our study), Bsharri (1) (our study), Tannourine (3) (our study), and Bsharri (9) [27] oil samples, defining consequently the predominance of α-pinene and β-pinene in these locations. Limonene, abieta-8,11,13-triene, and abieta-7,13-diene were identified in all samples at variable concentrations. It is noticeable that limonene, abieta-8,11,13-triene, and α-pinene were the major compounds of Adana, Turkey, oil sample (8) [24]. The oil composition of Başkonuş District, Turkey (7) [25], although dominated by α-pinene, exhibited the highest contents of abieta-7,13-diene and then characterized by α-pinene and abieta-7,13-diene majority.

The second PC is mainly correlated to myrcene, exclusively detected in Qartaba (5), Hadath Eljebeh–Tannourine reserve (6) [8] and then Adana, Turkey (8) [24], Bsharri (9) [27], and Başkonuş District, Turkey (7) [25]. This compound was checked as the most abundant in Qartaba samples (5) and was also found in significant concentration in Hadath Eljebeh–Tannourine reserve (6) [8]. The oils collected from these two regions were distinguished from the others by their remarkable content of myrcene. Therefore, the prevalence of myrcene, α-pinene, and limonene was marked in Qartaba (5), and α-pinene and myrcene in Hadath Eljebeh–Tannourine reserve (6) [8].

In summary, we pointed out that the comparison of our results with those reported exhibited the occurrence of several EO types according to the region of harvest and provided a new insight into the chemical variability of cedar cones EOs. These results clearly indicate that

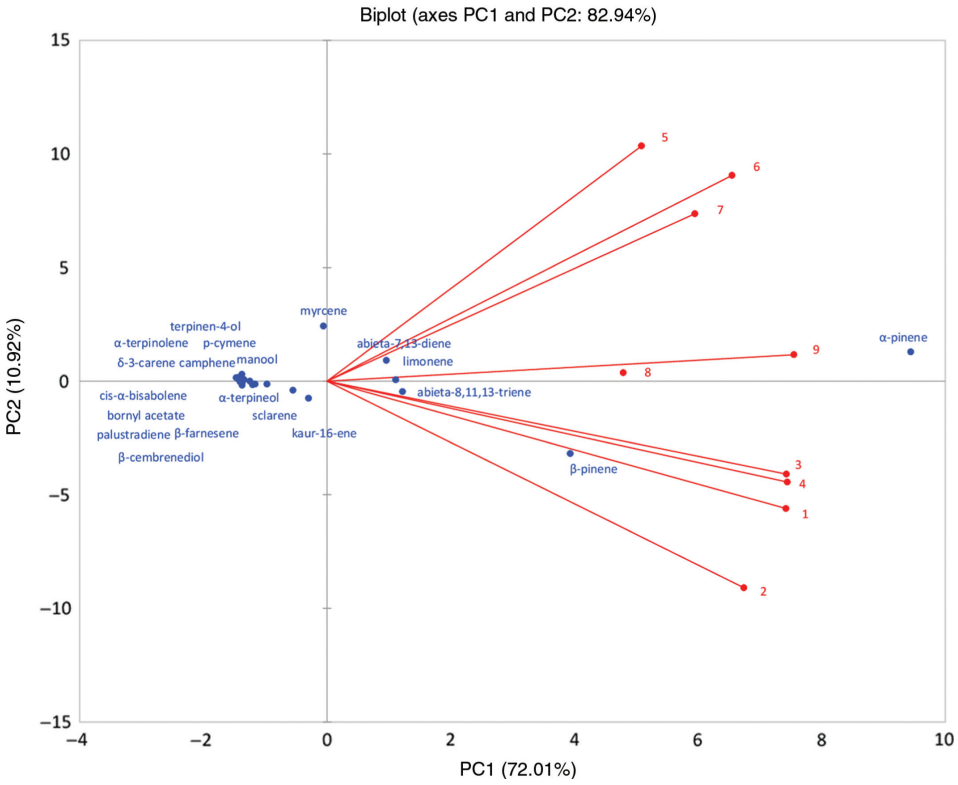

Figure 1: Principal component projection plot of PC1 and PC2 scores and loadings based on GC/MS data of cones EOs of *C. libani* collected from several geographical locations (our results and data extracted from literature). The numbers represent the samples collected from the different studied locations (our samples and data previously published). (1): Bsharri (our study), (2): Ehden (our study), (3): Tannourine (our study), (4): Chouf (our study), (5): Qartaba (our study), (6): Hadath Eljebeh–Tannourine reserve [8], (7): Başkonuş District, Turkey [25], (8): Adana, Turkey [24], (9): Bsharri [27].

biosynthesis of EO secondary metabolites is strongly influenced by geographical conditions: environmental factors of the different studied sites and cultivation conditions. Geographical location is associated to a number of environmental factors, such as precipitation, wind exposure, light intensity, UV radiation, high temperature and drought conditions, cold, frost, soil composition and fertility, and so on. The combination of all these factors puts pressure on the plants, with a need to develop mechanisms to be protected and to prevent damages. In fact, terpenoids are involved in specific adaptation strategies, such as defense against abiotic stresses and defense against pests and microorganisms [28]. Therefore, the variability observed in the accumulation of EO chemical compounds according to the region of harvest could be explained by the fact that specific compounds are mainly biosynthesized in a particular region in order to secure and protect the plant [13, 14, 18, 29]. Furthermore, this variability could be attributed to both abiotic and abiotic factors. These results stress the importance of selecting a harvesting region ensuring the optimal EO chemical composition that best fits the market demands.

3.2.4 Chemical variability according to cedar parts and extraction methods based on existing literature survey

According to previous reports, sesquiterpenes characterized mainly wood EO of the biblical cedar of Lebanon. Therefore, Saab et al. [30] in 2012 evidenced the predominant presence of β- (21.4%), α- (9.5%), and γ-himachalene (6%) isomers together with himachalol (5.6%), γ-(*Z*)-atlantone (5%), and allohimachalol (4.1%).

Başer and Demirçakmak in 1995 proved that *C. libani* wood EOs from Antalya and İçel were, respectively, distinguished by the dominance of β- (38.2%–34.3%), α- (12.8–11.5%), and γ-himachalene (7.6%–6.8%) isomers with *trans*-α-atlantone (7.8%–14.8%), himachalol (1.2%–8.8%), *trans*-β-atlantone (1.4%–2.4%), β-cedrene (1.8%–2.8%), allohimachalol (1.8%–2.3%), (*E*)-10,11-dihydroatlantone (3.7%–0.02%), *cis*-β-atlantone (1.1%–2.5%), and *cis*-α-atlantone (1.06%–2.1%) [5]. Saab et al. [3] in 2005 demonstrated the predominance of himachalol (43.1%) and γ- (11.9%), α- (7.12%), and β- (7%) isomers of himachalene and allohimachalol (3.92%) in *C. libani* wood EO. In

addition, they determined smaller amounts of the (*E*) and (*Z*) isomers of α-, β-, and γ-atlantone. According to Loizzo et al. [9] in 2008, himachalol (22.5%), β-himachalene (21.9%), α-himachalene (10.5%), and γ-himachalene (9.1%) were considered as peculiar of cedar wood EO.

Cedrus libani root EOs from İçel were characterized by the presence of β- (27.5%), α- (10.3%), and γ-himachalene (7.1%) isomers with *trans*-α-atlantone (23.7%), himachalol (9.7%), *trans*-β-atlantone (2.3%), β-cedrene (1.1%), allohimachalol (2.2%), (*E*)-10,11-dihydroatlantone (0.1%), *cis*-β-atlantone (2.3%), and *cis*-α-atlantone (2.8%) [5].

Limonene (17.7%) was the main compound in the EO obtained from the cones of *C. libani* natively grown in Turkey, followed by abieta-8,11,13-triene (17%) and α-pinene (12.3%). Limonene is used as an antimicrobial inhibitor in the food industry [24]. The chemical profile of EO from the oleoresin of the cones of *C. libani* grown in Turkey was distinguished by the preponderance of α-pinene (24.8%), abieta-7,13-diene (16.7%), abieta-8,11,13-triene (6.9%), manool (5.8%) and terpinen-4-ol (3.7%), α-terpineol (3.4%), p-cymene (2.9%), and limonene (2.7%) [25]. The investigation of the phytochemical composition of *C. libani* ethanol extract obtained from cones illustrated the dominance of α-pinene (51%), β-myrcene (13%), 7,13-abietadiene (3.2%), terpinolene (3.1%), and limonene (2.3%) [9].

Manool (11.0%) predominated in the hydrodistilled EOs of the *C. libani* trunk-bark samples [31].

Saab et al. [32] in 2011 reported that the main components of *C. libani* seed chloroform extracts were α- and β-pinene (34.4% ± 1.22% and 33.3% ± 1.08%, respectively). The occurrence of fatty acids and ethers as most abundant compounds, in particular oleic acid (17.3% ± 1.34%), methyl oleate (7.8% ± 0.42%), and ethyl oleate (5.3% ± 0.48%), in *C. libani* seed ethanol extract has been evidenced. Diterpenes, as neoabietol (11.8% ± 0.98%), abieta-7,13-diene (8% ± 0.46%), abieta-8(14), 13(15)-diene (7.9% ± 0.66%), abietol (6% ± 0.55%), and abietal (2% ± 0.13%), were also detected [32, 33].

For what concerns cedar leaves ethanol extract, germacrene D was checked as the most abundant compound (29.4%) [9]. It was found that fenchone (14.2%), β-thujone (28%), α-thujone (20.3%), camphor (8.7%), 1-borneol (4.7%), and endobornyl acetate (4%) were the main constituents in cedar leaf EOs [34].

Based on a PCA, a comparison of the main components (> 5%) of *C. libani* extracts, extracted from different parts (wood, roots, leaves, trunk-bark, seeds, and cones) and by different extraction methods (hydrodistillation, steam distillation, chloroform, and ethanol extraction), assessing our results and those previously published, is illustrated in Figure 2. Two PCs were chosen representing more than 62.29% of the variability. Principal component analysis results revealed the presence of three groups.

Cedar cones EOs, extracted by hydrodistillation (12, 13, 14, 15, 16) (our study), (17) [8], (18) [25], (19) [24], and (20) [27], and seed chloroform extract samples (10) [32] distributed in a group were clearly separated from the two other groups by the first principal component (PC1). These samples constituting the first cluster possessed in common high relative amounts of α-pinene, β-pinene, and then abieta-8,11,13-triene, myrcene, limonene, sclarene, palustradiene, and kaur-16-ene. α-Pinene and β-pinene are known to have antibacterial, antioxidant, anti-inflammatory, and anticarcinogenic activities [10, 18, 35–41].

A clear separation of the second cluster representing oils extracted from cedar wood (1, 2) [5], (3) [30], (4) [3], and (5) [9] and roots (6) [5] from the first cluster (cedar cones EOs and seed chloroform extract samples) is shown along PC1. This second cluster is separated from the third one by PC2 and characterized by high amounts of β-himachalene, α-himachalene, γ-himachalene, *trans*-α-atlantone, and himachalol. Principal component analysis allowed us to draw the following conclusions: cedar wood and root EOs had identical main components, and the relative concentration of these components was similar by both extraction methods: steam distillation and hydrodistillation.

The third cluster was separated from the group of seed chloroform extract and cedar cones EO samples by PC1 and from the group of wood and root EO samples by PC2. This cluster contained cedar leaves ethanol extract (7) [9], cedar leaves EO (8) [34], cedar EO from trunk-bark (9) [31], and cedar seed ethanol extract (11) [32] samples and was dominated by β-thujone, α-thujone, camphor, fenchone, β-caryophyllene, germacrene D, δ-cadinene, *trans*-α-bisabolene, 1-epi-cubenol, isolongifolene, manool, methyl oleate, oleic acid, ethyl oleate, abietol, neoabietol, abieta-7,13-diene, and abieta-8(14),13(15) diene.

According to PCA, seed ethanol extraction and seed chloroform extraction affected the chemical composition of seed extracts given the fact that different compounds were recovered after these two extraction methods.

These findings shed light on the cedar extract chemical polymorphism. Given the fact that each part of the same plant does not possess the same enzymatic equipment, the metabolomic profile is strongly dependent on plant parts or organs [42]. Extraction methods, extrinsic conditions, and interspecific and intraspecific factors also influenced plant extract chemical composition [14–16, 43–45].

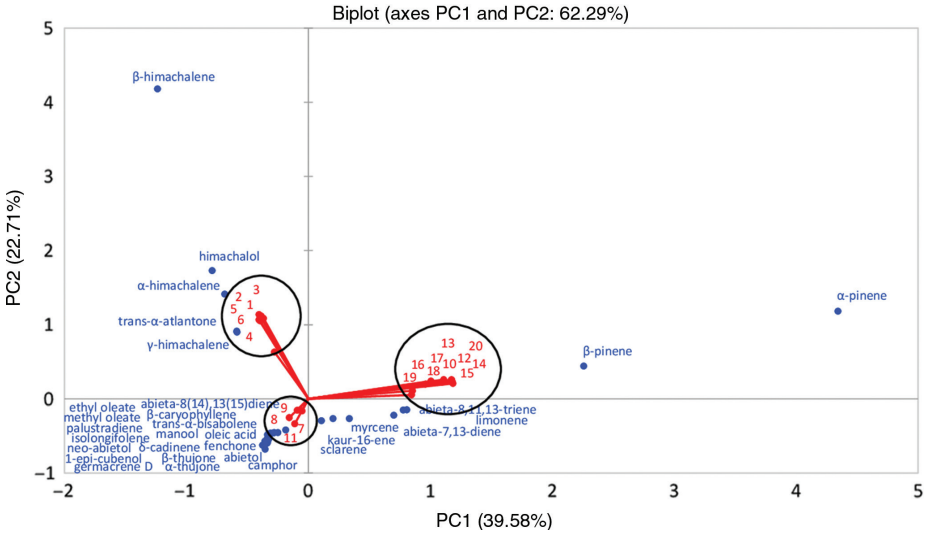

Figure 2: Principal component projection plot of PC1 and PC2 scores and loadings based on GC/MS data of *C. libani* extracts extracted from different parts (our results and data previously published). The numbers represent the samples extracted from the different studied *C. libani* parts (our samples and data previously published). (1): *C. libani* wood EO from Antalya, steam hydrodistillation [5]; (2): *C. libani* wood EO from Tarsus, steam hydrodistillation [5]; (3): *C. libani* wood EO, hydrodistillation [30]; (4): *C. libani* wood EO, hydrodistillation [3]; (5): *C. libani* wood EO, hydrodistillation [9]; (6): *C. libani* root EO from Tarsus, steam hydrodistillation [5]; (7): *C. libani* leaves ethanol extract [9]; (8): *C. libani* leaves EO [34]; (9): *C. libani* trunk-bark EO [31]; (10): *C. libani* seed chloroform extract [32]; (11): *C. libani* seed ethanol extract [32]; (12): *C. libani* cones EO from Bsharri, hydrodistillation (our study); (13): *C. libani* cones EO from Ehden, hydrodistillation (our study); (14): *C. libani* cones EO from Tannourine, hydrodistillation (our study); (15): *C. libani* cones EO from Chouf, hydrodistillation (our study); (16): *C. libani* cones EO from Qartaba, hydrodistillation (our study); (17): *C. libani* cones EO, hydrodistillation [8]; (18): *C. libani* cones EO, hydrodistillation [25]; (19): *C. libani* cones EO, hydrodistillation [24]; (20): *C. libani* cones EO, hydrodistillation [27].

4 Conclusion

Bearing in mind that *C. libani* is the sacred tree constituting the symbol of Lebanon, the results of this study contribute to a scientific valorization of this majestic tree anchored in beliefs and traditions. This first report bringing to light the chemical variability of EOs from the cones of cedars growing wild at four Lebanese natural reserves and protected areas (Bsharri, Chouf, Ehden, and Tannourine) and from cones of a cultivated cedar growing in Qartaba evidenced the existence of polymorphism in relation to the geographical location.

In light of this observation, our results affirm the variability of *C. libani* cones EOs depending on the geographical location and suggest the involvement of abiotic and biotic factors. Knowing that a better understanding of EO chemical variability in response to abiotic pressures is capital in the perspective of identifying new bioactive molecules and envisaging pharmacological activities of plants, our findings can be at the service of the determination of the best harvest locations in order to optimize growing and harvest conditions for optimal EO quality and quantity according to the market requirements.

In addition, a review of the existing literature on *C. libani* extracts composition allowed us to draw a relationship between chemical composition and cedar part, on the one hand, and chemical composition and extraction method. Further variability studies conducted on extracts of wild and cultivated *C. libani* according to seasonal variations, genetics, and soil composition should be considered in future investigations to further explore the chemical polymorphism.

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