EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2026, 27(3), 301–317
https://doi.org/10.12912/27197050/21830
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Received: 2026.01.25
Accepted: 2026.02.24
Published: 2026.03.01

Provenance-driven variation in yield and chemical composition of *Myrtus communis* L. essential oils across Moroccan biogeographic zones

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ABSTRACT

It was investigated the influence of provenance on the yield and chemical composition of *Myrtus communis* L. essential oils (EOs) across Morocco. A total of 143 samples were collected from twelve populations representing three distinct biogeographic zones: the pre-Rif, the Western Rif, and the Central Plateau. Essential oil yields varied significantly, ranging from 0.25% ± 0.13 in the IKA population (Western Rif) to 0.60% ± 0.20 in the BS population (Central Plateau), with the highest average yields recorded in populations from the Central Plateau (sub-humid zone). EOs were analyzed by gas chromatography-mass spectrometry (GC-MS), identifying 40 compounds that accounted for 93.7–99.98% of the total composition. Major constituents included 1,8-cineole, myrtenyl acetate, α-pinene, D-limonene, α-terpineol, linalool, methyleugenol, and geranyl acetate. Single-factor analysis of variance (ANOVA) and Pearson correlation analyses were used to assess the effects of provenance on both EO yield and composition. Multivariate analyses, including principal component analysis (PCA) and hierarchical cluster analysis, identified four distinct chemotypes across the natural distribution of *M. communis* in Morocco.These results demonstrate that provenance significantly affects both the quantity and chemical composition of myrtle essential oils, providing a quantitative basis for selecting populations with optimal EO profiles for medicinal, aromatic, and industrial applications.

Keywords: *Myrtus communis* L., essential oil, chemical composition, climatic factors, Morocco.

INTRODUCTION

Common myrtle (*Myrtus communis* L.) is an aromatic and medicinal plant that enjoys a strong reputation in the Mediterranean region owing to its therapeutic, cosmetic, and culinary properties (Özkan and Güray, 2009; Wahid, 2013; Aabdousse et al., 2020). Its wide range of applications is mainly attributed to its astringent, antiseptic, anti-inflammatory, antimicrobial, and antioxidant activities (Gortzi et al., 2008; Wahid, 2013; Aleksic and Knezevic, 2014; Bouaziz et al., 2015; Raeiszadeh et al., 2018; Sanna et al., 2019). The essential oils extracted from this species are particularly valued for their high quality in the aromatherapy and perfumery industries (Ammar et al., 2010).

Owing to their growing interest, essential oils (EOs) derived from aromatic and medicinal plants (MAPs) are increasingly considered an alternative and valuable source of bioactive compounds widely used in the medical, therapeutic, cosmetic, agri-food, and agricultural sectors (Bnouham et al., 2006; Zoubiri and Baaliouamer, 2011; Bakha et al., 2018; Ju et al., 2018). The effectiveness of EOs is primarily linked to their beneficial properties, which arise from their complex and diverse chemical composition.

Thus, myrtle essential oils are characterized by the predominance of α-pinene, 1,8-cineole, myrtenyl acetate, limonene, and linalool as major constituents (Flamini et al., 2004; Ebrahimabadi et al., 2016; Fadil et al., 2017). Indeed, myrtle EOs have been the subject of numerous studies conducted in several Mediterranean countries, including Morocco (Farah et al., 2006; Satrani et al., 2013; Harassi et al., 2019), Algeria (Moghrani and Maachi, 2008; Berka-Zougali et al., 2010), Tunisia (Jamoussi et al., 2005; Ammar et al., 2010), Spain (Boelens and Jimenez, 1990, 1991), and France (Bradesi et al., 1997; Chalchat and Garry, 1998).

The findings of these studies revealed considerable variability in both essential oil yield and phytochemical composition. Such variations are primarily attributed to multiple factors, including genetic background and environmental conditions, which may act independently or synergistically (Kofidis et al., 2003; Teuscheur et al., 2005; Stefanini et al., 2006; Crocoll et al., 2010; Lamamra, 2018).

However, the growing demand for this genetic resource by the phytotherapy, cosmetic, and pharmaceutical industries has compelled Moroccan myrtle producers to position themselves within a competitive international market, notably by increasing the availability of productive and stable varieties of this species. Owing to its numerous applications and significant benefits, investigating the variability and optimizing the yield of myrtle essential oil have become essential for the sustainable exploitation of this resource.

Furthermore, evaluating the influence of environmental conditions on both the quality (chemical composition) and quantity (yield) of essential oils extracted from aromatic and medicinal plants (MAPs) contributes to enriching databases used for modeling the stability of EO production (Bakhy et al., 2014; Aissi et al., 2016; Wahid et al., 2016; Fadil et al., 2017; Bakha et al., 2018).

Indeed, the phytochemical profile and chemical composition of essential oils can be used in combination with morphological and molecular traits to assess the intraspecific variability of aromatic and medicinal plants (Sacchetti et al., 2007; Lopez et al., 2008; Wahid et al., 2016; Fadil et al., 2017). Nevertheless, studies addressing the variation in yield and chemical composition of myrtle essential oils in Morocco remain limited and fragmentary, and they do not encompass all biogeographic areas where natural populations of the species occur. Most existing research has focused on specific biogeographic regions (Wahid et al., 2016; Fadil et al., 2017; Harassi et al., 2019).

Within this context, the present study represents the first comprehensive investigation aimed at evaluating both the quantitative (yield) and qualitative (chemical composition) variability of myrtle essential oils using samples collected across the entire biogeographic distribution of the species in Morocco.

MATERIALS AND METHODS

Plant material

Myrtle samples were systematically collected between November and January following the protocol described by Wahid et al. (2018). The sampling strategy was designed to encompass all biogeographic zones within the natural distribution range of the species. In total, 144 individuals were sampled, with each sample corresponding to a single genotype, from 12 populations distributed across three distinct biogeographic zones: the Western Rif, the pre-Rif, and the Central Plateau. These regions represent the major part of the natural distribution of myrtle in Morocco (Fig. 1).

Within each population, between nine and nineteen trees were randomly selected for leaf collection (Table 1). Variations in the number of sampled individuals among populations were related to differences in population size and spatial extent (Wahid et al., 2018). After collection, the plant material was placed in paper bags and transported to the laboratory, where it was air-dried under ambient conditions.

Extraction of essential oils

The myrtle leaves were shade-dried and subsequently subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus (Clevenger, 1928). For each distillation, 100 g of dried leaves were used. Essential oil yields were calculated as a percentage according to the formula reported by Marion et al. (1994), expressed as the weight of essential oil (g) per 100 g of dry plant material:

$$EO\ yield\ (\%) = \frac{Weight\ of\ the\ EO\ obtained\ by\ distillation\ (g)}{Weight\ of\ dried\ biomass\ (g)} \times 100 \quad (1)$$

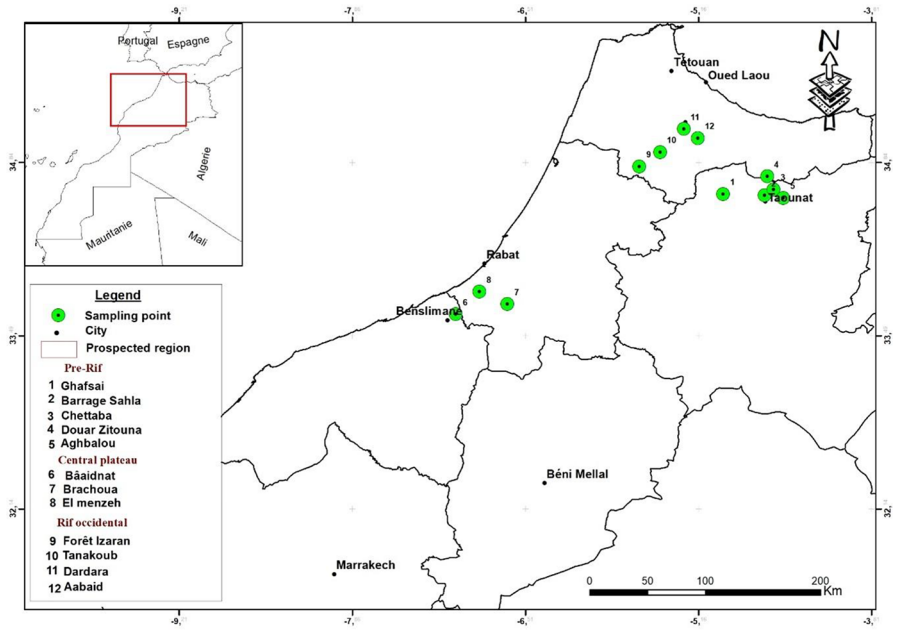

Figure 1. Sample sites of the natural myrtle populations studied

Table 1. Geographical and climatic characteristics of the sampling sites and the number of samples by population (N)

Population	Code	N	Ecological zones	Longitude	Latitude	Altitude (m)	Pr (mm)	T (°C)	Bioclimatic stage
Ghafsai	GHA	11	Pre-rif	34°35′ 39.8″N	04°57′ 59.5″W	441	772	18.1	Sub-humid
Barrage sahla	SAH	11		34°35′ 02.1″N	04°38′ 37.7″W	460	653	17.7	Sub-humid
Chettaba	ZRI	11		34°37′ 46.8″N	04°34′ 17.4″W	380	608	18	Sub-humid
Douar zitouna	IKA	11		34°43′ 53.4″N	04°37′ 19.8″W	475	654	17.2	Sub-humid
Aghbalou	AGH	9		34°33′ 47.6″N	04°29′ 47.1″W	439	595	17.5	Sub-humid
Bâaidnat	BS	11	Plateau central	33°39′ 28.4″N	07°02′ 59.7″W	275	470	16.7	Sub-humid
Kourifla	BRA	19		33°49′ 57.7″N	06°51′ 57.2″W	220	469	17	Sub-humid
El Menzeh	RAB	11		33°44′ 10.8″N	06°38′ 52.9″W	332	484	17.2	Sub-humid
Forêt Izaran	IZA	13	Rif Occidental/ Nord	34°48′ 29.0″N	05°37′ 06″W	411	742	18.5	Humid
Parc Bouh-achem	BOUH	15		34°55′25.6″N	05°27′27.9″W	285	871	17.7	Humid
Centre Ikejioun	DAR	10		35°06′11″N	05°16′23″W	450	805	16.9	Humid
Aabaïd	BT	11		35°01′48″N	05°09′43″W	745	984	15.9	Humid

The resulting essential oil was stored in dark glass vials, hermetically sealed, and kept at 4 °C until further analysis.

GC/MS analysis and identification of essential oil components

The myrtle essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC/MS) at the National Centre for Scientific and Technical Research of Morocco (CNRST-Morocco), within the Unit of Technical Support to Scientific Research (UATRS). Analyses were performed using a TRACE 1300 gas chromatograph coupled to a TSQ 8000 Evo mass spectrometer. Separation was achieved on a VB-5 capillary column, with helium as the carrier gas at a flow rate of 1.2 mL·min^{-1}. The transfer line and ion source temperatures were set at 250 °C and 200 °C, respectively. Injections were carried out in split mode, with an injected volume of approximately 1 µL using a split injector

maintained at 220 °C. Electron impact ionization was applied at 70 eV. For chromatographic analysis, the essential oils were diluted in ethanol at a ratio of 1:10 (v/v).

The components of the essential oils were identified based on their retention indices, determined relative to homologous series analyzed under the same GC conditions, and by comparing their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007).

Statistical analysis

Descriptive statistics [mean, standard deviation (SD), and coefficient of variation (CV)] were calculated for essential oil yield (%). The sampling design included twelve natural populations distributed across three Moroccan biogeographic zones (pre-Rif, Central Plateau, and Western Rif), with N = 143 individual samples in total (population sample sizes ranging from n = 9 to n = 19 (Table 1). Differences in EO yield among populations were tested using one-way analysis of variance (ANOVA) with population (12 levels) as a fixed factor. In addition, the effect of biogeographic zone (3 levels) on EO yield was evaluated using one-way ANOVA based on population means. Prior to ANOVA, assumptions were assessed on model residuals: normality was evaluated using the Shapiro-Wilk test and Q–Q plots, and homogeneity of variances was tested using Levene's test. When ANOVA was significant (α = 0.05), Tukey's HSD post-hoc test was applied for pairwise comparisons. The effect of provenance on essential oil chemical composition was assessed by performing one-way ANOVAs on the relative abundances (%) of major constituents across populations. Relationships between EO yield, major EO constituents, and ecological variables (e.g., altitude, mean annual precipitation, and mean annual temperature) were examined using Pearson's correlation coefficient (r), with statistical significance set at $p < 0.05$. Multivariate analyses were conducted to explore patterns in EO profiles among populations. Principal component analysis (PCA) was performed using EO yield and the relative abundances of 11 major compounds with mean contents higher than 0.5% across all samples (Table 8). Variables were standardized (z-scores) prior to PCA. Population grouping was further investigated using hierarchical cluster analysis (HCA) computed on standardized variables using Euclidean distances and Ward's linkage method, and resulting clusters were interpreted as chemotypes. Univariate analyses were performed in IBM SPSS Statistics v20.0 (IBM Corp., 2011), while PCA/HCA and graphics were produced in *R* v3.6.0 (R Core Team, 2017) using the packages FactoMineR, factoextra, stats, and ggplot2.

RESULTS

Characterization of EO yields of natural populations of myrtle in Morocco

Essential oil yield varied strongly among the twelve natural populations, revealing a clear provenance-related pattern across Morocco (Table 2). At the individual-sample level, EO yield ranged from 0.09 to 0.89%, while population mean yields ranged from 0.25 ± 0.13 (IKA) to 0.60 ± 0.20 (BS). Overall variability was high (total CV = 47.32%), indicating substantial heterogeneity among populations, which is also illustrated by the box-plot (Fig. 2). Marked intra-regional differences were observed as well: in the Central Plateau, EO yield ranged from 0.36 ± 0.16 (BRA) to 0.60 ± 0.20 (BS); in the Western Rif, from 0.30 ± 0.14 (DAR) to 0.44 ± 0.20 (BT); and in the pre-Rif, from 0.25 ± 0.13 (IKA) to 0.41 ± 0.14 (AGH).

The presentation of median EO yields of natural myrtle populations from the different geographic regions in a box plot further highlights this variation (Fig. 2). with an overall inter-population variation of approximately 47.32%. Intra-population variation also contributed significantly to the observed differences among populations. For instance, a coefficient of variation (CV) of 27.32% was recorded in the GHA population of the Pre-Rif. while the highest intra-population variability (CV = 65.45%) was observed in the IZA population from the Western Rif.

Characterization of the chemical composition of EOs from natural populations of myrtle in Morocco

GC/MS analysis of myrtle essential oil samples collected from natural populations in Morocco identified 40 chemical components. representing 93.72–99.98% of the total oil composition (Table 3). Considering the total chemical composition by population. the EOs were predominantly

Table 2. Descriptive statistics on the yield of myrtle essential oils

Population	Minimum	Maximum	Mean	Standard deviation	CV
DAR	0.12	0.50	0.30	0.14	46.66
BT	0.22	0.89	0.44	0.20	46.86
BOUH	0.11	0.83	0.37	0.20	53.93
IZA	0.17	0.83	0.35	0.23	65.45
GHA	0.22	0.57	0.36	0.10	27.32
SAH	0.18	0.53	0.31	0.11	33.67
AGH	0.22	0.61	0.41	0.14	34.54
IKA	0.09	0.47	0.25	0.13	51.05
ZRI	0.22	0.58	0.41	0.12	28.60
BRA	0.17	0.83	0.36	0.16	45.79
RAB	0.19	0.55	0.32	0.12	38.59
BS	0.36	0.88	0.60	0.20	32.93
Total	0.09	0.89	0.37	0.18	47.32

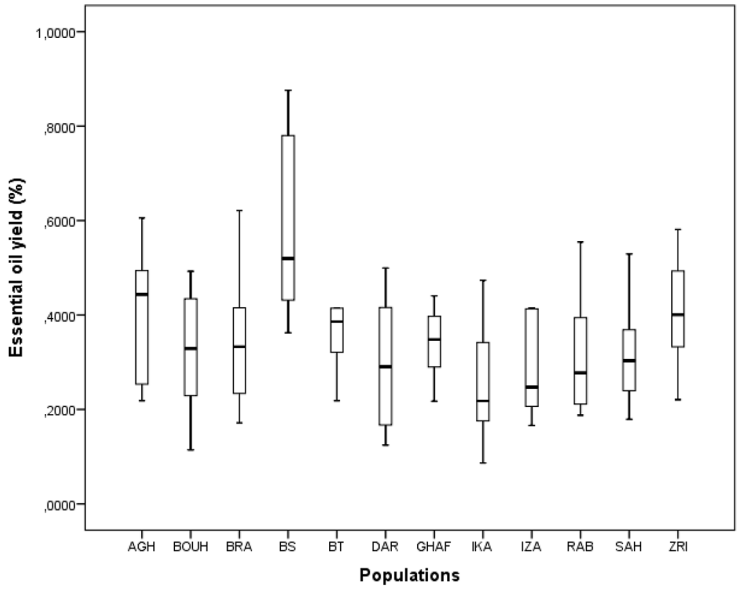

Figure 2. Box plot of essential oil yield

rich in oxygenated monoterpenes (59.95–82.91%) and monoterpene hydrocarbons (11.60–31.87%). In contrast. lower amounts of sesquiterpene hydrocarbons (0.18–1.53%) and oxygenated sesquiterpenes (0.99–4.35%) were observed.

The main constituents of these essential oils were 1.8-cineole (24.50–39.12%), myrtenyl acetate (8.53–36.32%), α-pinene (6.48–21.03%). D-limonene (4.99–11.02%), α-terpineol (2.18–6.63%), linalool (1.04-9.03%), methyleugenol (1.31–4.34%), and geranyl acetate (not detected – 6.12%) (Table 4).

Considerable variation in the levels of major essential oil constituents was observed among the populations studied. α-Pinene content ranged from 9.24% (BS) to 21.03% (BRA) in the Central Plateau, from 9.36% (BT) to 17.70% (DAR) in the Western Rif, and from 6.48% (ZRI) to 20.24% (AGH) in the Pre-Rif. D-limonene varied between 6.40% (BS) and 10.35%. BRA in the Central Plateau from 7.15% (BT) to 11.02% (DAR) in the Western Rif, and from 4.99% (ZRI) to 9.65% (AGH) in the pre-Rif.

For 1.8-cineole concentrations ranged from 25.60% (RAB) to 36.48% (BS) in the Central Plateau, from 30.19% (BOUH) to 33.81% (DAR) in the Western Rif, and from 24.50% (GHAF) to 31.12% (SAH) in the pre-Rif. In contrast, myrtenyl acetate exhibited the highest levels in the Western Rif ranging from 22.77% (DAR) to

Table 3. Chemical composition of the EO of the populations studied

Ordre	Component			Central plateau			Western Rif				Pre-rif				
		SI	RSI	BRA	RAB	BS	BT	BOUH	IZA	DAR	AGH	GHAF	IKA	SAH	ZRI
Monoterpene hydrocarbons															
1	α-Pinene	933	936	21.03	17.47	9.24	9.36	9.67	11.92	17.70	20.24	14.19	12.46	12.40	6.48
2	Camphene	822	914	0.06	0.05	0.05	0.03	0.03	0.03	0.05	0.05	0.06	0.03	0.03	0.02
3	Decane	948	958	0.08	0.04	0.03	0.08	0.05	0.07	0.05	0.09	0.07	0.03	0.07	0.03
4	Isobutyl 2-methylbutyrate	847	938	0.36	0.36	0.23	0.16	0.21	0.21	0.17	0.19	0.37	0.22	0.22	0.08
5	D-Limonene	914	914	10.35	9.75	6.40	7.15	9.51	9.19	11.02	9.54	9.65	8.86	7.43	4.99
Oxygenated monoterpenes															
6	1.8-cinéol	945	945	27.60	25.60	36.48	29.58	30.19	31.47	33.81	34.11	24.50	31.23	39.12	35.96
7	α-Terpinene	857	901	0.15	0.12	0.07	0.07	0.07	0.04	0.11	-	0.12	0.05	0.17	0.06
8	Nerol	664	758	0.36	0.59	0.54	0.13	0.62	2.80	0.12	0.55	3.70	0.31	3.35	0.36
9	α-Terpinolene	839	888	0.23	0.20	0.11	0.04	0.04	0.05	0.03	0.06	0.15	0.11	0.13	0.09
10	Linalool	913	915	3.16	9.03	10.72	2.21	1.09	1.86	1.04	2.68	2.69	7.28	5.64	8.52
11	trans-γ-Caryophyllene	823	915	-	0.07	0.07	0.03	0.03	-	-	0.02	-	0.05	0.05	0.05
12	α-Campholenal	863	908	0.08	0.05	-	0.03	0.03	0.03	0.05	0.03	0.03	0.03	-	0.03
13	Isoborneol	749	783	-	0.21	0.33	0.37	-	-	-	0.25	0.29	0.27	0.26	0.28
14	trans-Pinocarveol	904	915	0.65	0.35	-	0.19	0.18	0.12	0.08	0.61	0.29	0.34	0.24	0.38
15	Dodecane	815	899	0.36	-	-	0.30	0.16	0.20	0.17	0.28	0.37	-	0.31	-
16	Terpinen-4-ol	864	869	0.33	0.37	0.46	0.29	0.21	0.17	0.19	0.21	0.31	0.38	0.32	0.54
17	p-Mentha-1(7).8-dien-2-ol	824	867	0.22	-	0.11	0.04	0.07	0.07	0.05	-	0.08	0.11	0.05	0.15
18	α-Terpineol	926	927	3.77	3.81	5.00	3.88	3.30	2.18	2.69	2.63	3.82	4.62	4.27	6.63
19	(-)-Myrtenol	879	885	0.50	0.48	1.15	0.87	0.41	0.55	0.46	0.18	0.92	0.58	0.44	1.56
20	trans-Carveol	747	834	0.11	-	-	-	0.08	-	-	0.06	0.09	-	-	-
21	Pulegone	922	923	-	-	-	-	-	-	-	1.50	-	-	-	-
22	Linalyl acetate	895	896	-	-	-	-	-	-	-	0.78	-	-	-	-
23	Geraniol	879	895	0.32	0.53	0.52	0.42	0.23	0.21	0.28	0.19	0.40	0.59	0.34	0.80
24	Isobornyl acetate	823	865	0.05	0.04	0.06	0.04	0.05	0.04	-	-	0.05	-	-	0.04
25	p-Menthane-1.2.3-triol	603	674	-	-	-	-	-	-	-	0.02	-	-	-	-
26	Myrtenyl acetate	956	957	8.86	9.28	8.53	32.91	36.32	34.33	22.77	12.56	25.58	15.29	10.43	17.91
27	Carvyl acetate	894	905	0.19	0.09	0.17	0.26	0.22	0.22	0.16	-	0.19	0.14	0.10	0.15
28	α-Terpineol acetate	943	947	2.14	2.73	1.83	0.85	-	0.26	1.05	2.43	1.35	1.43	1.24	1.84
29	Eugenol	861	898	-	-	0.30	-	-	-	-	0.14	-	0.43	2.78	-
30	Geranyl acetate	955	956	6.11	6.12	4.22	3.60	2.96	-	3.91	3.78	-	4.87	-	4.28
31	trans-Myrtanyl acatate	879	885	0.82	-	1.23	-	-	-	-	0.45	-	-	-	-
32	trans-Pinocarvyl acetate	705	792	-	-	-	-	-	-	-	0.14	-	-	-	-
33	Methyleugenol	934	944	3.95	4.34	3.90	2.78	1.51	1.31	2.41	2.46	3.91	3.50	3.42	3.31
Sesquiterpene hydrocarbons															
34	Caryophyllene	878	903	0.89	0.97	-	0.30	0.17	0.19	0.27	0.39	0.51	0.45	0.36	0.33
35	β-Bisabolene	792	869	0.07	-	-	0.09	0.09	0.06	0.05	-	0.08	0.06	0.05	-
36	Humulene	844	913	0.34	0.56	0.18	0.10	0.13	0.09	0.12	0.19	0.22	0.29	0.17	0.20
Oxygenated sesquiterpenes															
37	Geranyl isobutyrate	710	863	0.24	0.15	-	0.19	0.16	0.11	0.17	0.04	0.20	0.19	0.10	0.17
38	Durohydroquinone	695	814	2.68	2.51	1.35	1.56	0.46	0.42	0.40	1.74	2.89	2.49	2.37	2.48
39	Asarone	680	729	0.29	0.22	0.13	0.36	-	-	0.18	0.12	0.22	0.17	0.14	0.16
40	Caryophyllene oxide	862	900	1.15	1.25	0.35	0.45	0.37	0.40	0.47	0.31	0.36	0.51	0.24	0.39

Monoterpene hydrocarbons (%	31.87	27.66	15.94	16.78	19.47	21.41	28.98	30.10	24.33	21.59	20.15	11.60
Oxygenated monoterpenes (%)	59.95	63.96	75.77	78.86	77.74	75.89	69.36	66.11	68.82	71.60	72.66	82.91
Sesquiterpene hydrocarbons (%	1.30	1.53	0.18	0.49	0.38	0.33	0.43	0.58	0.80	0.80	0.58	0.53
Oxygenated sesquiterpenes (%)	4.35	4.12	1.84	2.56	0.99	0.94	1.21	2.20	3.66	3.37	2.85	3.21
Total (%)	97.46	97.26	93.72	98.69	98.58	98.57	99.98	98.99	97.61	97.35	96.23	98.25

Table 4. Descriptive statistics of the majority components of myrtle essential oils

Components	Plateau central				Rif Occidental				Pre-Rif			
	Min	Mean	Max	Standard deviation	Min	Mean	Max	Standard deviation	Min	Mean	Max	Standard deviation
α-Pinene	9.24	15.91	21.03	6.05	9.36	12.16	17.70	3.86	6.48	13.15	20.24	4.92
D-Limonene	6.40	8.83	10.35	2.13	7.15	9.22	11.02	1.59	4.99	8.09	9.65	1.95
1.8-cinéol	25.60	29.89	36.48	5.79	29.58	31.26	33.81	1.87	24.50	32.98	39.12	5.54
Nerol	0.36	0.50	0.59	0.12	0.12	0.92	2.80	1.28	0.31	1.65	3.70	1.71
Linalool	3.16	7.64	10.72	3.96	1.04	1.55	2.21	0.58	2.68	5.36	8.52	2.65
α-Terpineol	3.77	4.19	5.00	0.70	2.18	3.01	3.88	0.74	2.63	4.39	6.63	1.46
Myrtenyl acetate	8.53	8.89	9.28	0.37	22.77	31.58	36.32	6.04	10.43	16.36	25.58	5.88
α-Terpineol acetate	1.83	2.23	2.73	0.46	0.26	0.72	1.05	0.41	1.24	1.66	2.43	0.49
Geranyl acetate	4.22	5.48	6.12	1.09	2.96	3.49	3.91	0.48	3.78	4.31	4.87	0.54
Méthyleugenol	3.90	4.06	4.34	0.24	1.31	2.00	2.78	0.71	2.46	3.32	3.91	0.53
Durohydroquinone	1.35	2.18	2.68	0.72	0.40	0.71	1.56	0.56	1.74	2.39	2.89	0.42

36.32% (BOUH). In the pre-Rif it varied from 10.43% (SAH) to 25.58% (GHAF), while populations in the Central Plateau displayed relatively lower values (8.53% in BS and 9.28% in RAB).

Effect of provenance on the yield and chemical composition of EO from natural populations of myrtle in Morocco

Myrtle plant material was collected from populations distributed across distinct biogeographic regions. and this variability is reflected in differences in EO yield among populations (Table 2). One-way analysis of variance revealed that the variance between populations exceeded the variance within populations (Table 5), indicating that population identity significantly influences myrtle EO yield in Morocco ($p < 0.001$). Furthermore, the sum of mean EO yields by region, followed by ANOVA assessing the effect of provenance, indicated that EO yield also varies across regions, although with lower significance compared to the effect of population (Table 5). These findings demonstrate that plant provenance has a significant impact on EO yield and identify populations that can be considered elite in terms of essential oil production.

Variability in the source of plant material was also reflected in the variation of chemical compound levels in the essential oils. One-way analysis of variance assessing the effect of provenance on EO chemical composition revealed highly significant differences ($p < 0.001$) in the levels of major constituents among the sampled populations (Table 6). These results allowed the identification of populations enriched in 1.8-cineole, α-pinene, and myrtenyl acetate, thereby enabling the determination of distinct chemotypes of myrtle essential oils in Morocco.

Phenotypic correlations

The essential oil yield of natural myrtle populations did not show significant correlations with ecological factors (Table 7). However, EO yield was positively and significantly correlated with 1.8-cineole content ($r = 0.62$; $P \leq 0.05$). A moderate negative correlation was observed between EO yield and myrtenyl acetate content ($r = -0.31$).

Analysis of the chemical composition of myrtle essential oils revealed significant correlations among the major constituents (Table 7). α-Pinene was strongly positively correlated with

Table 5. One-way ANOVA of the effect of origin on essential oil yield

Population	Populations/Region	Sum of squares	ddl	Mean of squares	F	Signification
By population	Inter-populations	0.919	11.000	0.084	3.145	0.000***
	Intra- populations	3.482	131.000	0.027		
By region	Inter- region	0.104	2.000	0.052	1.694	0.187
	Intra- region	4.297	140.000	0.031		

Table 6. One-way ANOVA of the effect of provenance on the chemical composition of myrtle EOs

Parameter	Populations	Sum of squares	ddl	Mean of squares	F	Signification
α-Pinene	Inter-populations	1 012.063	11	92.006	3.681	0.001***
	Intra-populations	899.750	36	24.993		
D-Limonene	Inter-populations	147.667	11	13.424	4.202	0.001***
	Intra-populations	115.000	36	3.194		
1.8-cinéol	Inter-populations	955.417	11	86.856	3.896	0.001***
	Intra-populations	802.500	36	22.292		
Nerol	Inter-populations	91.229	11	8.294	18.957	0.000***
	Intra-populations	15.750	36	0.438		
Linalool	Inter-populations	509.229	11	46.294	13.413	0.000***
	Intra-populations	124.250	36	3.451		
α-Terpineol	Inter-populations	68.500	11	6.227	2.893	0.008**
	Intra-populations	77.500	36	2.153		
Myrtenyl acetate	Inter-populations	4 864.667	11	442.242	25.473	0.000***
	Intra-populations	625.000	36	17.361		
α-Terpineol acetate	Inter-populations	24.650	10	2.465	7.728	0.000***
	Intra-populations	9.250	29	0.319		
Geranyl acetate	Inter-populations	36.222	8	4.528	3.327	0.009**
	Intra-populations	36.750	27	1.361		
Methyleugenol	Inter-populations	49.229	11	4.475	4.268	0.000***
	Intra-populations	37.750	36	1.049		
Durohydroquinone	Inter-populations	46.167	11	4.197	4.954	0.000***
	Intra-populations	30.500	36	0.847		

D-limonene (r = 0.86; $P \leq 0.01$) and negatively correlated with α-terpineol (r = -0.59; $P \leq 0.05$). Moderate correlations were also observed between α-pinene and α-terpineol acetate (r = 0.40), durohydroquinone (r = 0.37), 1.8-cineole (r = -0.33), linalool (r = -0.23), and myrtenyl acetate (r = -0.27). D-limonene was negatively correlated with α-terpineol (r = -0.69; $P \leq 0.05$), 1.8-cineole (r = -0.55), and linalool (r = -0.41). A strong negative correlation was observed between 1.8-cineole and durohydroquinone (r = -0.57), as well as between nerol and geranyl acetate (r = -0.57). Linalool exhibited significant correlations with multiple compounds: α-terpineol (r = 0.74; $P \leq 0.01$), myrtenyl acetate (r = -0.76; $P \leq 0.01$), methyleugenol (r = 0.76; $P \leq 0.01$), α-terpineol acetate (r = 0.70; $P \leq 0.05$), geranyl acetate (r = 0.54), and durohydroquinone (r = 0.35). α-Terpineol showed moderate correlations with myrtenyl acetate (r = -0.36) and methyleugenol (r = 0.49). Myrtenyl acetate was strongly negatively correlated with α-terpineol acetate (r = -0.79), methyleugenol (r = -0.72), and geranyl acetate (r = -0.60). Additionally, α-terpineol acetate correlated positively with geranyl acetate (r = 0.71), methyleugenol (r = 0.69), and durohydroquinone (r = 0.50). Geranyl acetate was also positively correlated with methyleugenol (r = 0.55), and a moderate correlation ($r \approx 0.69$) was observed between methyleugenol and durohydroquinone.

Correlation analysis revealed significant relationships between certain essential oil

Table 7. Correlation between EO yields, chemical composition and ecological factors

	Altitude (m)	*Pr* (mm)	*T* (°C)	EO yield	α-Pinene	D-Limonene	1.8-cinéol	Nerol	Linalool	α-Terpineol	Myrtenyl acetate	α-Terpineol acetate	Geranyl acetate	Methyleugenol	Durohydroquinone
Altitude (m)	1														
Pr (mm)	**.608***	1													
T (°C)	-.056	.109	1												
Rdt	.112	-.182	.095	1											
α-Pinene	.014	-.245	-.077	.007	1										
D-Limonene	-.161	-.028	-.049	-.224	**.860****	1									
1.8-cinéol	.070	-.196	.011	**.622***	-.329	-.545	1								
Nerol	-.210	-.070	**.712****	.252	.035	.056	-.098	1							
Linalool	-.273	**-.727****	-.112	.252	-.231	-.413	.168	.049	1						
α-Terpineol	.105	-.203	-.186	.245	**-.594***	**-.692***	.259	-.196	**.741****	1					
Myrtenyl acetate	.357	**.895****	.411	-.315	-.266	-.021	-.245	.084	**-.755****	-.364	1				
α-Terpineol acetate	-.343	**-.839****	-.165	.077	.406	.147	-.035	-.091	**.692***	.224	**-.790****	1			
Geranyl acetate	-.366	**-.599***	-.502	-.239	.197	.155	-.155	-.570	.542	.275	**-.606***	**.711****	1		
Methyleugenol	-.245	**-.615***	-.242	0.000	.210	.133	-.364	.084	**.755****	.490	**-.720****	**.692***	.549	1	
Durohydroquinone	.007	-.273	.267	-.238	.371	.266	-.573	.238	.350	.203	-.217	.503	.275	**.685***	1

constituents and ecological factors of the sampled populations (Table 7), indicating that variation in chemical composition is partly influenced by environmental conditions such as rainfall. temperature, and altitude. Nerol was strongly positively correlated with the temperature of the provenance ($r = 0.71$; $P \leq 0.01$). Myrtenyl acetate showed high to moderate positive correlations with precipitation ($r = 0.90$; $P \leq 0.01$), altitude ($r = 0.36$), and temperature ($r = 0.41$). In contrast, Linalool ($r = -0.73$; $P \leq 0.01$), α-terpineol acetate ($r = -0.84$; $P \leq 0.01$), methyleugenol ($r = -0.62$; $P \leq 0.05$), and geranyl acetate ($r = -0.60$; $P \leq 0.05$) were negatively correlated with precipitation. Additionally, geranyl acetate exhibited moderate negative correlations with temperature ($r = -0.50$) and altitude ($r = -0.37$).

Analysis of the structure of variability in yield and chemotypes of EOs

The principal component analysis was performed using essential oil yield and the relative abundances of 11 major compounds with mean contents higher than 0.5% across all samples (Table 8), namely 1.8-cineole, α-pinene, myrtenyl acetate, linalool, D-limonene, α-terpineol acetate, geranyl acetate, α-terpineol, methyleugenol, and durohydroquinone. The first two principal components (PC1 and PC2) accounted for a substantial proportion of the total variance (68.38%; Fig. 3). The distribution of populations according to the levels of major compounds was strongly associated with the first principal component (PC1 = 42.07%), which was positively correlated with α-terpineol acetate, methyleugenol, durohydroquinone, linalool, and geranyl acetate. and negatively correlated with myrtenyl acetate content. In contrast, PC2 was positively associated with α-terpineol and linalool and negatively associated with α-pinene and D-limonene. Overall, these results enabled the identification of four distinct chemical groups (chemotypes) of myrtle essential oils in Morocco.

Considering the geographical distribution of the four identified myrtle chemotypes, the 1.8-cineole chemotype was the most prevalent among populations from the Central Plateau, with a limited occurrence of the α-pinene chemotype. Furthermore. the myrtenyl acetate/1.8-cineole chemotype was detected in four populations from the Western Rif (BOUH, BT, IZA, and DAR) as well as in the GHA population from the pre-Rif Mountains. These populations were generally characterized by a high abundance of myrtenyl acetate. except for the DAR population. which was distinguished by a predominance of 1.8-cineole.

In contrast. the fourth chemotype, characterized by a combination of 1.8-cineole, myrtenyl acetate, and linalool, exhibited a more specific

Table 8. Loadings of morphological traits on the first two PCs (the highest one (>0.5 threshold) are in bold

Parameter	Component	
	1	2
α -Pinene	0.058	**0.936**
D-Limonene	-0.408	**0.865**
1.8-cinéol	0.095	-0.588
Nerol	-0.295	-0.186
Linalool	**0.844**	-0.338
α -Terpineol	**0.728**	-0.593
Myrtenyl acetate	-0.852	-0.239
α -Terpineol acetate	**0.845**	0.414
Geranyl acetate	**0.588**	0.395
Methyleugenol	**0.878**	0.161
Durohydroquinone	**0.751**	0.119
EO yield	0.095	-0.519

distribution pattern. This chemotype was present across all areas of the natural distribution of myrtle. although with marked variability in the relative proportions of its constituent compounds. resulting in different compositional profiles among regions (Fig. 4).

Figure 5 illustrates the corresponding dendrogram. while the mean and range (min-max) percentages of the major compounds according to provenance are presented in Tables 3 and 4.

Group I comprises essential oils from populations characterized by high myrtenyl acetate contents (32.91–36.32%), including the IZA. BOUH. and BT populations from the Western Rif. Among these, the BOUH population exhibited the highest level of this compound. This group is also distinguished by relatively high proportions of 1.8-cineole (29.58–31.47%). In contrast, populations belonging to this group showed low levels of α-terpineol acetate.

Group II includes essential oils from the GHA population of the pre-Rif and the DAR population of the Western Rif, both characterized by high levels of 1.8-cineole (24.50% and 33.81%, respectively). The second major constituent in this group is myrtenyl acetate, with concentrations ranging from 22.77% to 25.85%. In addition, this group is distinguished by relatively high α-pinene contents, varying between 14.19% and 17.70%.

Group III comprises essential oils from the RAB and BRA populations of the Central Plateau and the AGH population of the Pre-Rif. These populations are characterized by the predominance of 1.8-cineole (25.60%, 27.60%, and 34.11%, respectively) and α-pinene (17.47%, 21.03%, and 21.24%, respectively) as the main compounds. This group also exhibits substantial levels of D-limonene (9.54–10.35%), myrtenyl acetate (8.86–12.56%), and geranyl acetate (3.78–6.12%).

Group IV corresponds to essential oils from the BS population of the Central Plateau and the SAH, IKA, and ZRI populations of the pre-Rif. These populations are characterized by a predominance of 1.8-cineole as the major compound

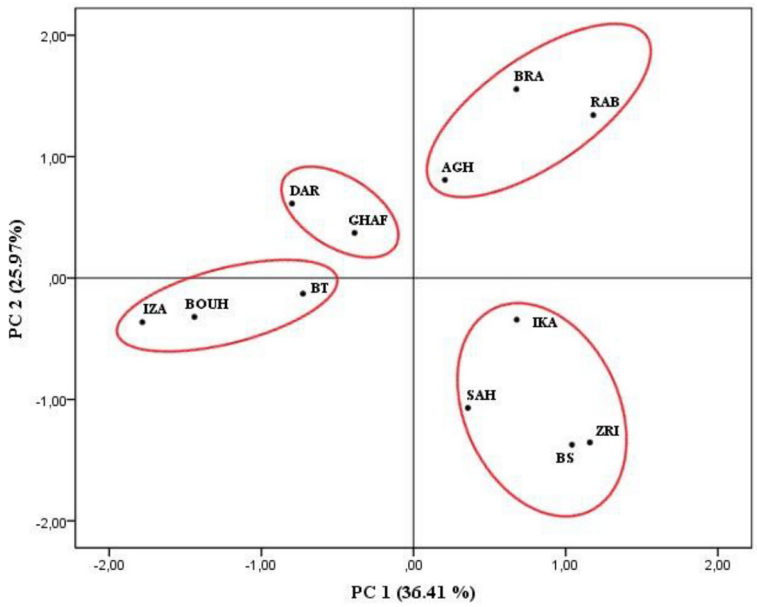

Figure 3. Component analysis of chemical variability in EO of natural populations of myrtle studied

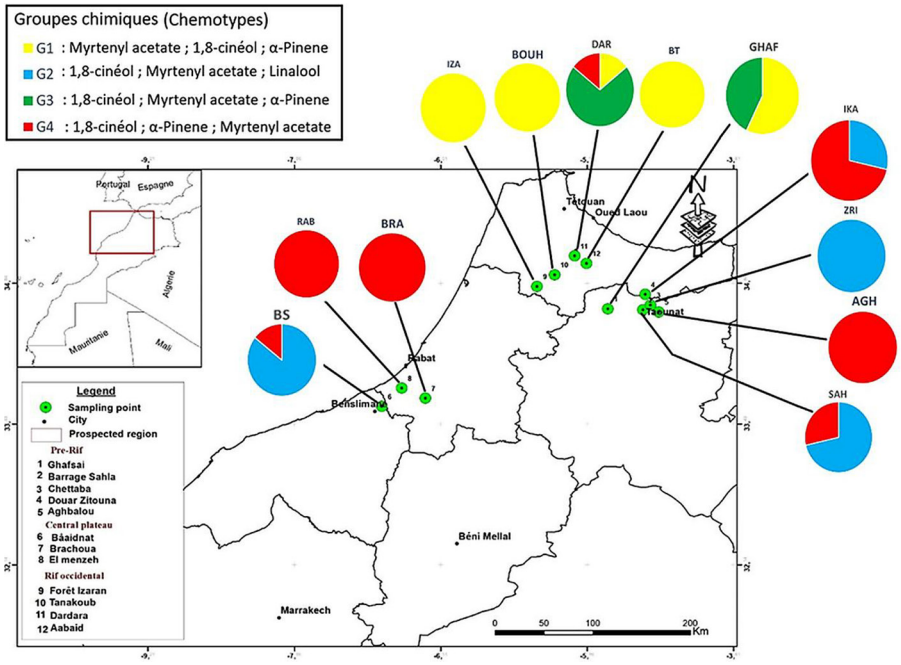

Figure 4. Distribution of essential oil chemotypes of *Myrtus communis* in natural populations

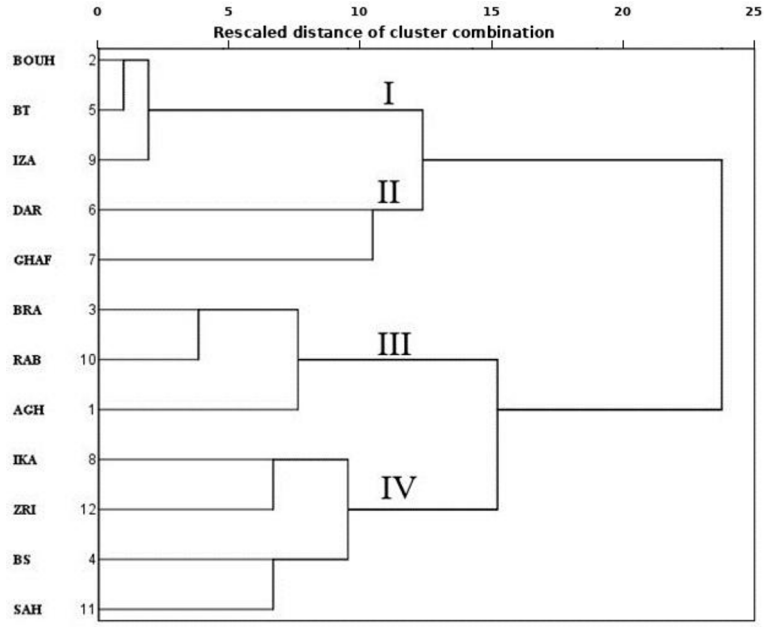

Figure 5. Hierarchical classification of natural populations of myrtle using Euclidean distance based on the chemical composition of EOs

(31.23–40.12%), with the highest content observed in the SAH population (40.12%). In the BS population, linalool represents the second major constituent (10.72%), whereas α-pinene is the second most abundant compound in the SAH population (12.40%). In contrast, the IKA and ZRI populations are characterized by myrtenyl acetate as the second major compound. with contents of 15.29% and 17.91%, respectively.

DISCUSSION

The importance of the quantity and nutritional quality of essential oils has been steadily increasing. Both national and international markets are becoming increasingly aware of the necessity to ensure the sustainable production and security of essential oils. Consequently, there is a growing need to establish new cultivation systems in order

to preserve the intrinsic characteristics of essential oils. as well as to develop new high-quality myrtle varieties. Understanding the variability of essential oil composition among natural myrtle populations is therefore essential. not only to support breeding programs. but also to ensure the conservation of genetic resources.

The present study aims to assess the effect of provenance on variations in yield and phytochemical profiles of myrtle essential oils originating from biogeographically distinct regions. Special emphasis is placed on elucidating the patterns of qualitative and quantitative variation of essential oils in relation to ecological conditions.

Characterization of variation in EO yield and chemical composition

Our results revealed a significant spatial variation in the essential oil yield of myrtle. Bioclimatic differences among the three regions. as represented by the twelve natural populations studied, appear to have a notable effect on EO content. The EO yield, calculated on a dry leaf basis. ranged from 0.25% to 0.60%. This finding is consistent with previous studies conducted in the same areas. although in a more fragmentary manner (Satrani et al. 2006; Wahid et al. 2016).

Conversely, other studies have reported relatively higher yields, ranging from 0.48% to 1.06% (Ghanmi et al. 2011; Fadil et al. 2016. 2017). At the Maghreb scale. EO yield values for myrtle in Algeria (0.32%) and Tunisia (0.61%) are comparable to those observed in Moroccan populations. Howeve, these values are lower than those reported in Italy (0.22–0.90%; Tuberoso et al. 2006) and Portugal (0.33–0.74%; Pereira et al. 2009). In contrast, myrtle populations in Greece and Iran exhibit higher EO yields than those recorded in Morocco. ranging from 1.20% to 1.45% and 0.53% to 1.75%, respectively (Gardeli et al. 2008; Rahimmalek et al. 2013).

The observed variation in essential oil yield among Moroccan natural populations of myrtle may be attributed to genetic factors at the time of plant material collection. as reported by Bradesi et al. (1997), and also to the vegetative stage. as indicated by Jamoussi et al. (2005), who found that maximum EO yield is generally achieved at the flowering stage. In addition to these intrinsic factors, environmental conditions of the provenance may also contribute to this variation, as demonstrated by Szakiel et al. (2010), Furthermore, environmental stresses have been shown to influence EO production (Sharma et al. 2012; Fadil et al. 2016).

In the present study. variation in environmental conditions is particularly evident. as the populations examined belong to distinct bioclimatic zones (humid and sub-humid) and biogeographic regions (Rif, pre-Rif, and Central Plateau). These findings indicate that the geographic origin of myrtle plant material in Morocco has a significant effect on EO yield.

The qualitative characterization of essential oils from natural Moroccan myrtle populations revealed a predominant composition comprising varying proportions of 1.8-cineole, myrtenyl acetate, α-pinene, D-limonene, α-terpineol, linalool, methyleugenol, and geranyl acetate. These findings are consistent with several previous studies investigating the chemical composition variability of myrtle EOs (Yadegarinia et al. 2006; Gardeli et al. 2008; Petretto et al. 2015).

In the present study, 1.8-cineole was the dominant compound (24.50–39.12%), followed by myrtenyl acetate (8.53–36.32%) and α-pinene (6.48–21.03%), in agreement with the results reported by Fadil et al. (2017). Conversely. other authors have reported α-pinene as the main constituent of myrtle EOs (Flamini et al. 2004; Bouzabata et al. 2013. 2015; Rahimmalek et al. 2013). Such differences in chemical composition may be attributed to environmental factors, genetic variability, or their interaction.

Regarding the qualitative variability of essential oils among natural Moroccan myrtle populations. our study demonstrates a significant effect of provenance. Myrtle populations from the Western Rif are characterized by a predominance of myrtenyl acetate (22.77–36.32%) and 1.8-cineole (29.58–33.81%). In contrast. populations from the pre-Rif and Central Plateau exhibit higher contents of 1.8-cineole (24.50–39.12% and 25.60–36.48%. respectively) and α-pinene (6.48–20.24% and 9.24–21.03%. respectively). and occasionally D-limonene (4.99–9.64% and 6.40–10.35%. respectively).

As noted by Bakha et al. (2018). geographical and climatic factors can influence the variability of EO biosynthetic processes. thereby affecting both the quality and quantity of essential oils. Several studies have further shown that the chemical polymorphism of myrtle EOs may result not only from geographical origin and environmental conditions. but also from genetic factors (Flamini

et al. 2004; Wannes et al. 2011; Rahimmalek et al. 2013). Additionally, Pereira et al. (2009) reported that the EO yield and composition are influenced by the vegetative cycle of the plant material.

Phenotypic correlations

This study revealed significant and positive correlations between EO yield and 1.8-cineole content (r = 0.62), indicating that some populations may exhibit both high EO yield and elevated 1.8-cineole levels. In contrast, a moderate negative correlation was observed between EO yield and myrtenyl acetate (r = -0.31), suggesting that high EO yield may coincide with lower proportions of myrtenyl acetate.

Moreover, a strong positive correlation was found between the percentage of myrtenyl acetate and precipitation (r = 0.96). Myrtle populations from the Western Rif, which experiences the highest rainfall. are rich in myrtenyl acetate but relatively low in EO yield. Conversely. populations from the Central Plateau and pre-Rif regions. characterized by moderate rainfall. display higher EO yields and 1.8-cineole content, but lower myrtenyl acetate levels.

Overall, EO yield did not show a direct correlation with ecological conditions, but it was negatively associated with myrtenyl acetate and indirectly related to precipitation. Several studies have similarly reported correlations between environmental factors and EO yield (Bakhy et al. 2014; Bakha et al. 2018). These results underscore the importance of considering the ecological conditions of myrtle provenances for the indirect selection of populations with the highest EO production potential.

In the present study, significant correlations among the chemical compounds of myrtle essential oils were observed. Selecting a population based on high content of a specific compound may influence the abundance of other compounds of economic or therapeutic interest. For instance, populations selected for high myrtenyl acetate content tend to be low in α-terpineol acetate (r = -0.79), methyleugenol (r = -0.72), and geranyl acetate (r = -0.60). Similarly, selecting populations based on high 1.8-cineole content is associated with lower levels of D-limonene (r = -0.55) and durohydroquinone (r = -0.57). Populations rich in α-pinene also exhibit high D-limonene content (r = 0.86). but low α-terpineol levels (r = -0.59).

The phenotypic correlations observed between myrtle EO compounds and ecological conditions indicate that precipitation is a key determinant for most chemical constituents. This suggests that the distribution of characteristic chemotypes in natural myrtle populations follows patterns of ecological variation in precipitation. Specifically, populations from the Western Rif develop in a humid climate with higher annual precipitation, whereas populations from the Central Plateau and pre-Rif zones grow in a subhumid climate, characterized by relatively higher average temperatures and moderate rainfall.

Yield structure and chemical composition of myrtle

The results of the principal component analysis and hierarchical clustering of myrtle populations. based on the major EO compounds – 1.8-cineole, myrtenyl acetate, α-pinene, D-limonene, linalool, and α-terpineol – enabled the classification of populations into chemical groups and the identification of four distinct chemotypes of Moroccan myrtle EOs. The myrtenyl acetate/1.8-cineole chemotype represents EOs from the Western Rif Mountain populations (BOUH, BT, IZA, DAR) and the pre-Rif GHA population. This chemotype was previously reported by Fadil et al. (2017) in the Taounate region (Sahla Dam). Among these. the DAR and GHA populations are particularly rich in 1.8-cineole relative to myrtenyl acetate, placing them in a separate cluster from BOUH, IZA, and BT. These populations predominantly occur in a humid bioclimatic stage. except for GHA, which is technically in a sub-humid zone, but local climatic factors justify its classification as humid. All these populations share high and comparable annual rainfall rates. The 1.8-cineole/α-pinene chemotype, with modest amounts of D-limonene and geranyl acetate, as reported by Bradesi et al. (1997) and Flamini et al. (2004). corresponds to the BRA and RAB populations of the Central Plateau and the AGH population of the pre-Rif. These populations exhibit similar mean annual temperatures and precipitation, justifying their clustering. The 1.8-cineole/linalool/α-pinene chemotype corresponds to the EOs of the pre-Rif SAH, ZRI, and IKA populations, as well as the Central Plateau BS population. These populations share moderate and comparable annual precipitation, supporting their classification within a sub-humid climate.

The attribution of different chemotypes to the essential oils of the studied myrtle populations can be explained by variations in the biosynthetic pathways of these specialized secondary metabolites across regions. These variations may result from intrinsic genetic factors, such as the expression of specific genes controlling physiological processes involved in adaptation to water and/or thermal stress, as well as oxidative stress (Selmar and Kleinwächter. 2013). Alternatively, they may be influenced by external factors. particularly the differing environmental conditions of the natural habitats of these populations.

Climatic variability is likely a major driver of chemotype differences. Natural myrtle populations are distributed across two main bioclimates. which differ in climatic factors such as precipitation. temperature, and humidity, creating a variety of microclimates in each zone. Populations from the pre-Rif and Central Plateau develop in subhumid climates, although precipitation rates differ between these regions. In contrast, Rif populations grow under a humid climate characterized by high rainfall and humidity.

In response to these environmental conditions. myrtle plants adjust their morphological, physiological. and biochemical traits, enabling them to maintain both growth and EO production in terms of quantity and quality (Rizhsky et al. 2002; Liu et al. 2005; Madhava Mittler. 2006; Rao et al. 2006; Li et al. 2010).

CONCLUSIONS

The results of the present study revealed significant quantitative and qualitative variability in myrtle essential oils across their natural distribution range. Indeed. the central plateau populations (0.36–0.60%) exhibited higher essential oil yields than those from the western Rif (0.30–0.44%) and the pre-Rif (0.25–0.41%). In addition. a marked chemical polymorphism was observed in the EO composition of the studied populations.

Based on the relative proportions of the major compounds, namely 1.8-cineole, myrtenyl acetate, α-pinene, D-limonene, linalool, α-terpineol, and geranyl acetate – four distinct chemotypes of Moroccan myrtle were identified. Principal component analysis and hierarchical cluster analysis allowed the classification of these chemotypes as follows: (I) the myrtenyl acetate/1.8-cineole chemotype, represented by the Western Rif populations (BOUH, BT, IZA, DAR) and the pre-Rif GHA population; (II) the 1.8-cineole/myrtenyl acetate chemotype, observed in the DAR (Western Rif) and GHA (pre-Rif) populations; (III) the 1.8-cineole/α-pinene/D-limonene/geranyl acetate chemotype, characterizing the BRA and RAB populations from the central plateau as well as the AGH population from the pre-Rif; and (IV) the 1.8-cineole/linalool/α-pinene chemotype, found in the SAH, ZRI, and IKA pre-Rif populations and in the BS central plateau population.

These results, together with the correlations observed between EO yield and chemical composition. clearly indicate that the origin of the plant material significantly influences both the qualitative and quantitative variability of myrtle essential oils in Morocco. Consequently, geographical, environmental, and climatic factors constitute key parameters for a better understanding and evaluation of essential oil biosynthesis in aromatic plants. which occurs under diverse ecological conditions.

The observed phytochemical richness of myrtle essential oils highlights their considerable potential for valorization in the agri-food and pharmaceutical sectors, as well as for applications in other industrial fields. Furthermore, the pronounced chemical profile polymorphism of myrtle EOs represents a valuable database for the selection of superior genetic material. which could be integrated into future breeding and improvement programs for this species.

Acknowledgements

The authors are thankful the Director of the National Centre for Scientific and Technical Research of Morocco and the head of the Technical Support Unit for Scientific Research (UATRS) and the staff of this unit for their collaboration in carrying out the GC/MS analyses.

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