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Effect of provenance on leaf morphological traits and secondary metabolite levels in leaf extracts of Myrtle (*Myrtus communis* L.) in Morocco

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Abstract

Myrtus communis L. is widely used as an aromatic and medicinal plant. In this work, we investigate the influence of provenance on leaf morphological traits and secondary metabolite levels in leaf extracts of Myrtle. Samples of myrtle from different geographically distinct areas were measured and analysed for polyphenols, flavonoids, condensed tannins, and antioxidant activity using the DPPH test. The analysis of result reveals the significant variance among populations in terms of morphological traits and levels of secondary compounds, particularly in terms of polyphenols, flavonoids, condensed tannins, and reducing power. Correlations between certain studied traits and environmental conditions were recorded. Leaf size and methanolic leaf extract yields from Moroccan myrtle showed as good criteria for natural population selection. Precipitation, temperature, and altitude were determinants of variation in leaf morphological traits, extract yield, and their polyphenolic, tannin, and flavonoid compounds. Based on the studied traits, three major groups were identified with few clear geographical affiliations. The IZA population from the Western Rif and GHA from the Pre-Rif formed a separate group from the other populations, characterized by the highest extract yields. The BRA population from the central plateau formed a distinct isolated group from the others. This population, collected from a location with low annual rainfall values was the richest in tannins with relatively high levels of polyphenols and flavonoids compared to other populations. This means that drought affects the amount of polyphenols and flavonoids. The geographical structure of the third group is mixed, including the IKA and AGH (Pre-Rif) populations, BS and RAB from the Central Plateau, and BT from the Western Rif.

Keywords: *Myrtus communis* L., leaf morphology, leaf extract yield, phenolic compounds, antioxidant activity, provenance effect.
Abbreviations: CE_Catechin equivalent; GAE_Gallic acid equivalent, DPPH_ α, α-diphenyl-β-picrylhydrazyl; IC_Concentration inhibition; GHA_Ghafsai population, IKA_Douar Zitouna population; AGH_Aghbalou population; BS_Bâaidnat population; BRA_Kourifla population: RAB_El Menzeh population; IZA_Forêt Izaran population; BT_Aabaid population; PC_ principal component.

Introduction

Common Myrtle (*Myrtus communis* L.) is a distinctive shrub of significant ecological and social importance in the Mediterranean basin (Wahid, 2013; 2018; Melito et al., 2016; Aabdousse et al., 2021). Its distribution spans across the entire Mediterranean basin to the Middle East and Asia (Zilkah and Goldschdmidt, 2014; Aabdousse et al., 2021). In Morocco, this aromatic and medicinal plant is found in three distinct biogeographical zones, varying from semi-arid to subhumid climates, encompassing the regions of Pre-Rif, Western Rif, and the Central Plateau (Aabdousse et al., 2021).

Furthermore, Myrtle is utilized not only as an ornamental plant (Agrimonti et al., 2007) but also commonly in traditional medicine, cosmetics, and the agro-food industry (Wahid et al., 2013). According to Aabdousse et al. (2020), Myrtle leaves are traditionally employed in Morocco to treat various ailments due to their therapeutic properties. Several studies have shown that the leaves of this shrub are a crucial source of antioxidants owing to their high content of phenolic compounds (Dellaoui et al., 2018; Bouchenak et al., 2020). Additionally, Myrtle fruits are rich in tannins, anthocyanins, and other antioxidant compounds (Fadda et al., 2015). However, the chemical composition of the plants can be influenced by environmental factors, especially pedoclimatic conditions, in combination with genetic factors (Ruiz-Rodríguez et al., 2014; Maieves et al., 2015).

Moreover, the domestication of Myrtle has been driven by increasing industrial demand for its leaves, fruits, and by-products. Climate change and the growing threat to agro-resources have promoted the expansion of Myrtle cultivation and the selection of elite populations rich in chemical compounds and morphological traits of economic and ecological interest. Populations resistant to drought with high yields of metabolic compounds are highly sought

after. However, domestication and genetic improvement programs rely on a rich database of genetic, morphological, ecophysiological, phytochemical, etc., variation in relation to the provenance and/or geographical effect, for the establishment of mother plant collections.

Nevertheless, the phytochemical characterization of methanolic leaf extracts of Myrtle and the evaluation of their antioxidant activity in different biogeographical zones in Morocco remain largely unknown. In this context, the objectives of this study are as follows: (i) to define the morphological and phytochemical profile of methanolic leaf extracts of Myrtle, (ii) to assess their antioxidant activity, and (iii) to examine the relationship between provenance effects, morphological characteristics, and the phytochemical profile of leaf extracts from natural Myrtle populations in Morocco.

Results

Variation in leaf morphological traits among studied populations

Table 1 presents the descriptive statistics of leaf morphological traits in Myrtle leaves collected from natural populations. The results demonstrate significant variations among populations in terms of length (LGFe), width (LRFe), and the LGFe/LRFe ratio ($P < 0.001$). Specifically, leaf length ranges from 3.67±0.64 cm for the AGH population in the Pre-Rif to 4.59±1.08 cm observed in the IZA population in the Western Rif, with a coefficient of variation (CV) of 9.57%. On the other hand, leaf width varies from 1.27±0.45 cm, the lowest value recorded in the AGH population, to 1.54±0.53 cm in the BS population in the Central Plateau. These variations in length and width also reflect on the leaf length-to-width ratio, which also exhibited significant variations among the studied populations. This could be attributed to genetic diversity and variations in environmental conditions at the sampling sites.

Yield of methanolic extract by population

Figure 1 presents the results obtained regarding the yield of leaf extracts from natural populations of Myrtle collected from various locations in Morocco. Analysis of variance (ANOVA) with a single factor (Table 2) reveals a significant difference in the yield of methanolic leaf extracts among the studied natural populations of Myrtle. This indicates that the geographical origin of the samples has an impact on the extract production. Specifically, the Izarane (IZA) population in the Western Rif exhibits the highest extract yield with an average value of 13.33% ± 1.36, followed by the Ghafsay (GHA) population in the Pre-Rif with a value of 12.08% ± 2.50, and the populations of Bab Taza (BT) and Rabat (RAB) with values of 10.00% ± 1.18 and 10.00% ± 2.36, respectively. Conversely, the Ikawen (IKA) population in the Pre-Rif displays the lowest yield (7.92% ± 1.59). The variation in the geographical origin of the samples is reflected in the extract yield variation among the studied populations.

Analysis of phytochemical profile related to natural populations in Morocco

The results of descriptive statistics and one-way analysis of variance (ANOVA) are presented in Table 2. A highly significant difference ($P < 0.05$) was observed among the various studied populations concerning the content of flavonoids, polyphenols, and condensed tannins present in the methanolic leaf extracts of Myrtle. These findings underscore the significance of provenance in the variation of secondary metabolite levels in *Myrtus communis* leaf extracts. This suggests that the observed variations in flavonoid, polyphenol, and condensed tannin levels may be attributed to the geographical origin of the studied Myrtle samples. These results imply that specific factors related to the region or environment, in which Myrtle grows, could influence the production of these chemical compounds in the leaves. It is noteworthy that the observed difference is highly significant, further validating the results and indicating a strong link between provenance and the chemical composition of Myrtle leaf extracts.

Polyphenol content

The total polyphenol content in Moroccan common myrtle leaves varied significantly among the studied populations (Table 2). Notably, the IZA population is considered the richest in polyphenols, with an average content of 429.95±43.01 mg GAE/g of extract, ranging from 384.70 to 487.20 mg GAE/g of extract, with a coefficient of variation (CV) of 10%. This is followed by the BRA population from the central plateau, which records an average value of 383.84±37.02 mg GAE/g of extract, with a minimum value of 332.24 and a maximum value of 419.10 mg GAE/g of extract (CV = 9.64%). However, the BS population from the central plateau exhibits the lowest polyphenol content, with an average of 207.675±107.40 mg GAE/g of extract and a coefficient of variation of 51.71%.

Flavonoid content

The flavonoid content in Moroccan *Myrtus communis* leaves varied significantly among the studied biogeographic zones (Table 2). Specifically, the BRA population from the central plateau exhibits a significant richness in flavonoids, with an average content of 146.36 ± 23.04 mg EC/g of extract. The flavonoid content in this population ranges from 113.8 to 165.2 mg EC/g of extract, with a coefficient of variation of 15.74%. The IZA population from the Western Rif followed the trend of high flavonoid content with a value of 129.75 ± 18.70 mg EC/g of extract, ranging from a minimum of 116.2 ± 23.04 mg EC/g of extract to a maximum of 157.3 ± 23.04 mg EC/g of extract, and a coefficient of variation of 14.41%. In contrast, the IKA population from the Pre-Rif exhibited the lowest flavonoid content with an average of 96.75 ± 10.13 mg EC/g of extract and a coefficient of variation of 10.47%. Concerning intra-population variation, the highest variation in flavonoids is recorded in the BS population from the central plateau, with a coefficient of variation of approximately 26%. Meanwhile, the GHA population registers the lowest intra-population variation with a CV of 1.42%.

Condensed tannin content

The content of condensed tannins in the methanolic leaf extracts of Moroccan myrtle also exhibits significant variation among the studied populations (Table 2). Specifically, the content of these compounds ranges from 115.3 ± 24.29 mg EC/g of extract in the IZA population, representing the lowest content, to 406.54 ± 44.99 mg EC/g of extract in the BRA population from the central plateau, indicating the highest richness in tannins. However, the RAB population recorded the highest intra-population variation rate with a CV of 54.62%, followed by the IKA population, where tannin content ranges from 179.6 to 462.4 mg EC/g of extract, with an average of 321.18 ± 133.7 mg EC/g of extract and a coefficient of variation of CV = 41.62%.

Table 1. Variation in morphological leaf traits of naturally collected myrtle populations in Morocco.

Trait Population	LGFe (cm)	LRFe (cm)	LRFe/LGFe
BRA	4.31±0.58 ab 3.10-8.50 (CV: 13.50)	1.50±0.48 a 0.92-5.90 (CV: 31.75)	0.35±0.12 0.15-1.48 (CV: 34.06)
BT	4.60±0.83 a 1.94-7.63 (CV: 18.06)	1.47±0.27 a 0.91-1.98 (CV: 18.27)	0.33±0.08 0.16-0.74 (CV: 25.84)
IZA	4.59±1.08 a 1.00-15.00 (CV: 23.46)	1.53±0.52 a 0.80-5.00 (CV: 33.93)	0.34±0.14 0.12-1.40 (CV: 39.89)
GHA	3.69±0.61 b 2.00-5.00 (CV: 16.48)	1.30±0.48 b 1.00-3.00 (CV: 36.83)	0.38±0.06 0.26-0.61 (CV: 15.35)
IKA	3.82±0.73 b 2.00-6.00 (CV: 19.06)	1.39±0.51 b 1.00-4.00 (CV: 37.05)	0.52±2.12 0.25-1.20 (CV: 22.80)
AGH	3.67±0.64 b 2.00-5.00 (CV: 17.52)	1.27±0.45 b 1.00-3.00 (CV: 35.83)	0.86±4.81 1.00-3.00 (CV: 24.08)
RAB	4.26±0.86 ab 1.42-6.77 (CV: 20.17)	1.45±0.41 ab 1.00-2.00 (CV: 28.43)	0.35±0.11 0.17-1.39 (CV: 29.90)
BS	4.53±0.92 a 2.00-9.20 (CV: 20.34)	1.54±0.53 a 1.00-5.90 (CV: 34.57)	0.35±0.14 0.14-1.48 (CV: 39.57)
Moyenne	4.18±0.40 1.00-15.00 (CV: 9.57)	1.43±0.10 0.80-5.90 (CV: 7.13)	0.40±0.18 0.12-1.48 (CV: 41.90)
F	**62.934*** **	**1.201****	**1.440****

BRA : Kourifla, BT : Aabaïd, IZA : Forêt Izaran, GHA: Ghafsai, IKA: Douar zitouna, AGH: Aghbalou, RAB : El Menzeh, BS : Bâaidnat. Traits' abbreviation: LGFe: Leaf length, LRFe: Leaf width, LRFe/LGFe: the ratio of leaf width and length. Abbreviation for descriptive statistics.

Conversely, the GHA population exhibited the lowest intra-population variation with a CV of 7.36%.

Antioxidant activity of myrtle leaf extracts

The antioxidant activity of the compounds is attributed to their reducing power, which is assessed using the DPPH assay. The antioxidant activity of methanolic leaf extracts from myrtle plants collected from eight different locations was determined based on the concentrations that provide 50% inhibition (IC50) compared to the IC50 of a reference antioxidant, specifically, ascorbic acid. The IC50 values were calculated from the regression curves in Figure 2 and are presented in Figure 3. The results in this figure show variation among the studied populations in terms of antioxidant power (IC50). The antioxidant activity (IC50) ranged from 34.03 ± 5.12 µg/ml for the BRA population from the central plateau, exhibiting the highest radical-scavenging power, while it was 62.51 ± 13.87 µg/ml for the IKA population from the Pre-Rif, showing the lowest antioxidant power (Figure 3). Thus, extracts from populations in the central plateau demonstrate significant antioxidant power compared to the power of ascorbic acid as a reference antioxidant (34.35 ± 1.28 µg/ml). Conversely, populations from the Pre-Rif region exhibit relatively lower antioxidant activity compared to other biogeographic zones. This difference in IC50 values for methanolic leaf extracts of myrtle across populations by provenance is supported by one-way analysis of variance ($P < 0.001$).

Correlation between leaf traits, phytochemical profiles of myrtle extracts, and environmental factors

Table 3. displays the correlations between the phytochemical profile of myrtle leaf extracts and the environmental conditions of the natural sites where this plant thrives. Leaf length is strongly correlated with leaf width (r = 0.93). These leaf morphological traits are negatively correlated with the temperature of the growth sites (LGFe: r = -0.51, LRFe: r = -0.46). In fact, as the site temperature increases, the leaf size becomes smaller. This explains that the size of myrtle leaves in Morocco is associated with temperature. Additionally, leaf size shows a negative correlation with the 50% inhibitory concentration (IC50%, LGFe: r = -0.6, LRFe: r = -0.46). Therefore, larger leaf size corresponds to higher reducing power.

The yield of methanolic leaf extracts shows relatively positive correlations with precipitation (r = 0.47), temperature (r = 0.42), and polyphenols (r = 0.55). This suggests that higher precipitation and temperature favours myrtle growth and may contribute to significant extract and polyphenol yields. However, the yield of methanolic leaf extracts shows a strong negative correlation with tannins (r = -0.79). Depending on the ecological site conditions, a moderate association is observed between polyphenols and temperature (r = 0.51), flavonoids and altitude (r = -0.59), and between tannins and precipitation (r = -0.44). This means that high levels of polyphenols, flavonoids, and tannins may be associated with sites that are respectively warm, at lower altitudes, and receive less precipitation. It can be said that the expression of each of the studied

Table 2. Descriptive statistics and F-statistic of polyphenol, flavonoid, and tannin compounds in methanolic leaf extracts of wild *Myrtus communis* in Morocco.

Population	Yield (%)	Polyphenols (mg EAG/ g of extract)	Flavonoides (mg EC/ g of extract)	Tannins (mg EC/g of extract)
IZA	13.33±1.36a11.67-15.00 CV: 10.21	429.95±43.01 a 384.7- 487.2 CV: 10.00	129.75±18.70 a 116.2-157.3 CV: 14.41	115.3±24.29 c 79.6-131 CV: 21.07
BRA	8.34±2.36 bc 6.67-11.67 CV: 28.28	383.84±37.02 a 332.24-419.1 CV: 9.64	146.36±23.04 a 113.8-165.2 CV: 15.74	406.54±44.99 a 343.9-451 CV: 11.06
GHA	12.08±2.50 ab 10.00-15.00 CV: 20.69	346.24±84.55 ab 223.8 - 418.1 CV: 24.42	108.7±1.55 ab 106.9 -110.7 CV: 1.42	245.82±18.09 bc 218.9 - 257.4 CV: 7.36
BT	10.00±1.18 b 8.33-10.83 CV: 11.79	303.17±35.64 abc 264.0-339.4 CV: 11.75	100.03±6.22 ab 93.4-106.4 CV: 6.21	243.5± 25.19 bc 218.1- 275.3 CV: 10.34
IKA	7.92±1.1.59 bc6.67-10.00 CV: 20.13	250.45± 71.25 bc 198.3-355.7 CV: 28.45	96.75±10.13 ab 85.0-109.7 CV: 10.47	321.18±133.7 ab 179.6-462.4 CV: 41.62
RAB	10.00±2.36 b 8.33-13.33 CV: 23.58	263.673±59.34 bc 197.9-341.5 CV: 22.50	106.698±4.98 ab 99.7-111.45 CV: 4.66	276.178±151.7 ab 161.7-498.1 CV: 54.93
BS	8.75±2.50 bc 6.67-11.67 CV: 28.56	207.675±107.40 bc 69.3-330.6 CV: 51.71	108.5±28.10 ab 72.4- 140.7 CV: 25.90	236.725±32.31 bc 193.9-272.4 CV :13.65
AGH	8.34±3.04 bc 5.00-11.67 CV: 36.51	346.7±22.76 ab 319.7-375.3 CV: 6.56	107.678±8.46 ab 99.3-117.7 CV: 7.86	284.038±100.43 ab 149.6-375.3 CV: 35.36
F-statistic (Probability)	3.16 (0.016**)	5.43 (0.001***)	4.62 (0.002***)	3.92 (0.005***)

BRA : Kourifla, BT : Aabaïd, IZA : Forêt Izaran, GHA: Ghafsai, IKA: Douar zitouna, AGH: Aghbalou, RAB : El Menzeh, BS : Bâaidnat.
Abbreviation for descriptive statistics:

secondary metabolites occurs under specific environmental stress conditions at the site.

Geographical structure of myrtle populations in relation to studied traits

Principal Component Analysis (PCA) was conducted based on the yield of methanolic leaf extracts and the content of secondary compounds in all studied populations. The first two principal components (PC1 and PC2) significantly explain the total variance in the data (63.36%, Figure 4). The first component, explaining 36.15% of the variance, is significantly correlated with the content of polyphenols and flavonoids. However, the second component is significantly correlated with yield and the content of condensed tannins, explaining 27.21% of the variance. These results lead to the formulation of three major groups of individuals with a tendency towards geographic distinctiveness (Figure 4).

It is observed that the groups are formed by individuals from the same geographic origins or localities with similar biogeographical conditions, except for some individuals characterized by biased values of certain secondary metabolite compounds (IKA4 and RAB1). Thus, individuals from the BRA population in the central plateau have formed a distinct isolated group from the other populations. Individuals from the IZA population in the Western Rif and GHA in the Pre-Rif have formed a separate group from the other populations and are characterized by the highest extract yields. On the other hand, individuals from the IKA and AGH (Pre-Rif), BS and RAB (Central Plateau), and BT (Western Rif) populations have been grouped together due to similarity in tannin content and IC50 value.

Discussion

The analysis of leaf size characteristics (length and width) and secondary metabolites from leaf extracts (yield, polyphenols, flavonoids, tannins, IC50, and antioxidant

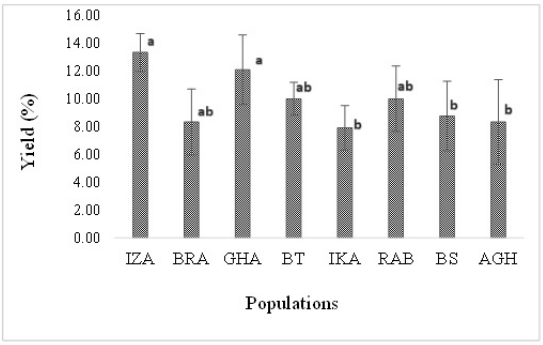

Figure 1. Yield of leaf extracts by natural populations of common myrtle in Morocco.

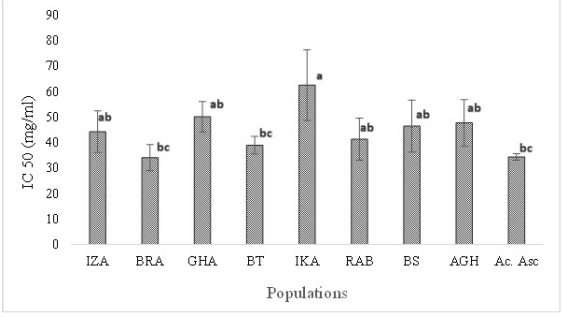

Figure 2 . Presentation of concentrations of methanolic leaf extracts of myrtle by natural population in Morocco in relation to percentage of inhibition.

Table 3. Correlation between morphological traits, phytochemical profile, and environmental factors in natural myrtle sites in Morocco.

	Altitude (m)	Pr (mm)	T (°C)	Rendement	Polyphénols	Flavonoides	Tanins	IC50	LGFe	LRFe
Altitude (m)	1									
Pr (mm)	0.919***	1								
T (°C)	-0.365	-0.186	1							
Rendement	0.174	0.470	0.420	1						
Polyphénols	-0.010	0.254	0.510*	0.544*	1					
Flavonoides	-0.588*	-0.344	0.243	0.175	0.688*	1				
Tanins	-0.301	-0.443	-0.320	-0.786**	-0.196	0.169	1			
IC50	0.156	0.076	0.278	-0.133	-0.368	-0.609*	-0.093	1		
LGFe	0.021	0.089	-0.514*	0.237	0.006	0.308	-0.353	-0.597*	1	
LRFe	-0.241	-0.157	-0.460	0.114	-0.067	0.415	-0.197	-0.459*	0.932***	1

Table 4. Geographic and climatic characteristics of sampling sites and sample number per 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
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	Central Plateau	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	Western Rif /North	34°48′ 29.0″N	05°37′ 06″W	411	742	18.5	Humid
Aabaïd	BT	11		35°01′48″N	05°09′43″W	745	984	15.9	Humid

N: Sample number per population natural, Pr: Precipitation, and T: Temperature.

activity) has demonstrated significant variability among natural populations of Myrtle. This variability in terms of the studied traits has clearly revealed that differences among populations are linked to the provenance effect (environmental and genetic). These results align with those observed in various natural Myrtle populations at the national scale (Mulas et al., 2002; Ruffoni et al., 2003; Messaoud et al., 2007; Barboni et al., 2010; Migliore et al., 2012; Sharma et al., 2012; Dahmoune et al., 2015; Fadil et al., 2016; Aabdousse et al., 2019). In fact, the variation among provenances could be explained by extreme differences in environmental conditions, such as precipitation, temperature, orography, and soil type, which characterize distinct biogeographic zones.

The yield of methanolic leaf extracts from Myrtle varies remarkably among the studied populations. This indicates that the origin of Myrtle plant material could have a significant effect on leaf extract yield (Sharma et al., 2012; Fadil et al., 2016). The Western Rif population (IZA) and the pre-Rif population (GHA) both exhibit high yields, reaching 13.5%. The IZA population (Western Rif), which achieved the highest yield, is characterized by a humid climate with annual precipitation rates exceeding 700 mm and average temperatures of 18.5°C. Meanwhile, the GHA population in the pre-Rif region, with a yield value of approximately 12.08%, features a sub-humid climate with precipitation levels and temperatures similar to those of the IZA population (772 mm/year and 18.1°C, respectively). These maximum yields observed in natural populations in Morocco are lower than those reported by other authors. For instance, Dellaoui et al. (2018) recorded a yield of 35.2%, while Touaibia and Chaouch (2013) found a yield of 35.56% for methanolic extracts of Algerian Myrtle. Hayder et al. (2008) also reported a yield of 28.66% for Tunisian Myrtle. The variability observed in the quantity of these extracts could be attributed to the polarity of the solvent used (Athamena et al., 2019) as well as intrinsic factors related to the specimens, such as genetics, vegetative stage, and collection period (Bradesi et al., 1997; Jamoussi et al., 2005; Yangui et al., 2017), and the existing differences in eco-climatic factors at the sampling sites (Fadil et al., 2016). Furthermore, the observed variability in both methanolic extract yield and chemical compounds among the studied zones could be explained by the variation in intrinsic plant factors, such as genetics, plant age, vegetative cycle, etc., as well as by extrinsic factors related to the environmental conditions at the sites, such as climate, edaphic factors, exposure, orography, altitude, etc. Additionally, the plant's adaptive capacity in response to these factors could influence the preferential biosynthesis of chemical compounds and their content, resulting in differential expression (Mansouri et al., 2011; Fadil et al., 2016).

The levels of secondary metabolites also exhibit variability among populations originating from different biogeographic zones. This suggests that provenance could be the source of this variability. Thus, the levels of polyphenols and flavonoids in Myrtle leaf extracts in Morocco are higher compared to other Mediterranean regions. For instance, Yangui et al. (2021) recorded polyphenol levels in Tunisian Myrtle leaf extracts ranging from 160.69±2.45 to 219.33±2.41 mg GAE/g extract, while flavonoids varied from 41.01±0.14 to 88.08±3.01 mg RE/g extract. On the other hand, studies on Algerian Myrtle have also shown lower polyphenol values compared to those noted for Moroccan Myrtle (Dahmoune et al., 2015). Conversely, other research on Myrtle from western and northern Algeria has reported values in terms of polyphenol, flavonoid, and condensed tannin content that are relatively similar to those observed in natural Myrtle populations in Morocco (Touaibia and Chaouch, 2013; 2015). However, the content of phenolic compounds or other secondary metabolites may depend on the polarity of the solvent used, the plant part subjected to extraction, the state of the plant material, and the extraction method employed (Athamena et al., 2019).

High variation within the population for leaf morphological traits is demonstrated in the present study. These results confirm differences in the genetic potential of genotypes. The observed variability within the population in terms of leaf morphological traits may provide an adaptability advantage to *Myrtus communis* populations in response to climate change (Wahid et al., 2016; Aabdousse et al., 2019).

Regarding the antioxidant capacity of methanolic leaf extracts from Moroccan Myrtle, we observed a significant difference among extracts from the studied populations. The extracts from the BRA population, located in the central plateau and characterized by high levels of tannins, polyphenols, and flavonoids, exhibit significant antioxidant activity. Additionally, samples from the BT population in the Western Rif, which has moderate levels of these compounds, also display remarkable antioxidant power. Conversely, the IKA population in the pre-Rif region exhibits low radical scavenging activity against DPPH compared to other populations, despite having moderate levels of tannins and polyphenols. This could be explained by the quality of phenolic compounds in its extracts. Several previous studies have demonstrated a strong correlation between the reducing capacity of extracts and the presence of these compounds (Keskes et al., 2014; Taviano et al., 2013; Lesjak et al., 2011). Furthermore, these compounds are used as scavengers of peroxide radicals and intermediate alkoxyl radicals, as well as chelating agents for metal ions, playing essential role in the initiation step of radical reactions. They are also recognized for their anti-inflammatory properties (Lesjak et al., 2011). Consequently, we can observe that the variation in the antioxidant activity of extracts from the studied areas could be explained by their richness in phenolic compounds and their quality. For this reason, we observed that populations rich in polyphenols exhibit significant reducing power. This implies that provenance, including geographical, edaphic, and climatic characteristics, could be responsible for the variation in secondary metabolite levels and, consequently, the biological potential of plant extracts (Mansouri et al., 2011).

In the present study, correlations were found between leaf morphological traits and secondary compounds in leaf extracts. Among the traits, leaf length is strongly correlated with leaf width (r= 0.93). Additionally, leaf size exhibits a

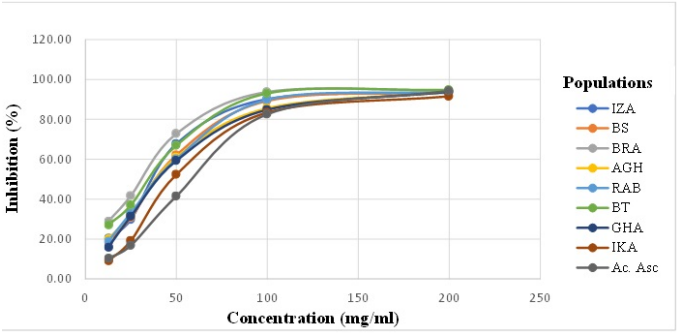

Figure 3. IC50 Values of methanolic leaf extracts from natural myrtle populations in Morocco.

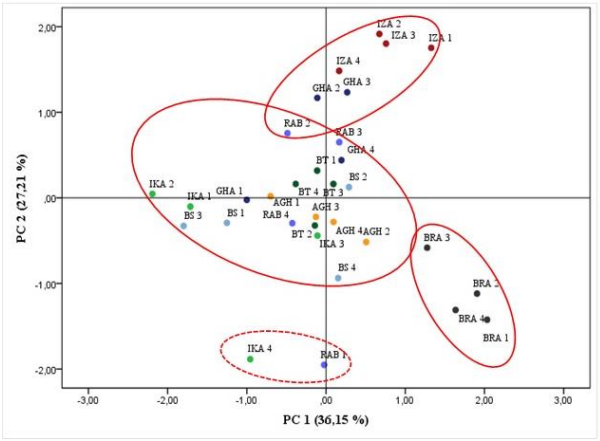

Figure 4. Principal component analysis of variability in yield of methanolic leaf extracts from common myrtle in Morocco and their polyphenol, flavonoid, and tannin content.

negative correlation with the half-maximal inhibitory concentration (IC50). These results indicate that selecting populations characterized by larger leaves may result in higher reducing power. Similarly, the yield of methanolic leaf extracts shows a negative correlation with tannins and a positive correlation with polyphenols. This suggests that recommending the selection of superior genotypes in the natural population based on the yield of methanolic leaf extracts will contribute to providing extracts rich in polyphenols and low in tannins. Therefore, leaf size characteristics and the yield of methanolic leaf extracts serve as good selection criteria for natural Myrtle populations in Morocco, meeting commercial demands and the goals of domestication and genetic improvement programs.

Environmental conditions appear to be important determinants of the traits and secondary compounds studied in the present study. In this regard, higher temperatures are associated with smaller leaf morphological traits, higher yield of methanolic leaf extracts, and increased phenolic compounds. Precipitation levels are critical for the presence or absence of tannins, while altitude influences the richness of methanolic leaf extracts in flavonoids.

The geographical structure of natural Myrtle populations in Morocco is determined through principal component analysis, based on morphological traits, secondary compounds, and environmental site conditions. Three major groups are defined in the present study. Our results show a compensation between metabolites in the studied extracts, with variation from one biogeographic zone to another related to temperature, precipitation, and altitude. For example, the IZA population from the Western Rif and the

GHA population from the Pre-Rif have formed a separate group from the other populations and are characterized by the highest extract yields. The BRA population from the central plateau forms a distinct isolated group from the others. This population, which experiences low annual precipitation, is the richest in tannins, with relatively high levels of polyphenols and flavonoids compared to other populations. The geographical structure of the third group is mixed, consisting of the IKA and AGH populations from the Pre-Rif, BS and RAB from the central plateau, and BT from the Western Rif.

Materials and Methods

Plant material

Leaf sampling was systematically conducted between December 2021 and February 2022. Eight natural populations of Myrtle were collected from different biogeographical regions of Morocco, including the pre-Rif, western Rif, and the central plateau (Figure 5). Table 4 summarizes the geographical and ecological characteristics of the various populations considered in this study. For each population, trees were randomly selected for leaf collection at mid-height from different exposures. The collected plant material was dried away from light and humidity at room temperature. Subsequently, it was carefully stored in paper bags, separated by tree, population, and geographical region, in a dry place for later use in the extraction process.

Preparation of extracts

The extraction was carried out following the protocol of Bouyahya et al. (2016). Maceration was performed using 3 grams of dried Myrtle leaf powder placed in a flask containing 20 ml of methanol at room temperature for 3 days with daily agitation. The plant extract was filtered through a filter paper (Whatman), and the methanolic solution was removed using a rotary evaporator to obtain a crude extract. The extracts were stored at 4°C until further use.

Determination of total polyphenol content

The quantification of total polyphenols was conducted following the Folin-Ciocalteu method (Singleton and Rossi, 1965), with modifications as described by Laouicha et al. (2020). In this procedure, 0.3 ml of the extract (at a concentration of 1 mg/ml) was mixed with 1.5 ml of Folin-Ciocalteu reagent for 4 minutes. Subsequently, 1.2 ml of 7.5% sodium carbonate was added. After one hour of incubation at room temperature, the absorbance was measured at 750 nm using a spectrophotometer. Three repetitions were performed for each tree and population. The concentrations of total phenolic compounds were expressed in micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg of extract).

Determination of flavonoid content

The quantification of flavonoids was conducted following the method described by Tenuta et al. (2002), involving the use of aluminium chloride (AlCl3) reagent and sodium hydroxide (NaOH). In this procedure, 250 µl of the extract (at a concentration of 1 mg/mL) was added to 1 ml of distilled water, followed by the addition of 75 µl of 5% (w/v) sodium nitrite (NaNO2). After 5 minutes of reaction, 150 µl of 10% (w/v) aluminium chloride (AlCl3) were added, and after 6 minutes, 500 µl of 1 M sodium hydroxide (NaOH) and 500 µl of 5% (w/v) sodium nitrite (NaNO2) were incorporated. The mixture was stirred and allowed to stand for 30 minutes at room temperature. Absorbance was measured at 510 nm. Three repetitions were performed for each tree and population. The total flavonoid content was expressed in micrograms of catechin equivalents per milligram of extract (µg CE/mg of extract).

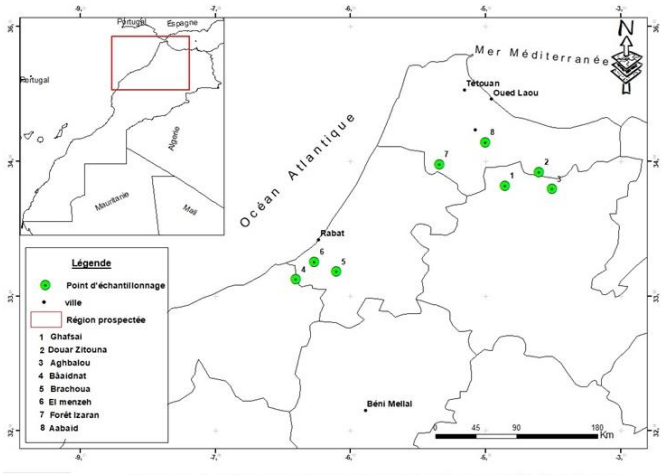

Figure 5. Sampling sites of natural myrtle populations studied in Morocco.

Determination of condensed tannin content

Condensed tannins were determined using the vanillin assay in an acidic medium, following the method described by Price et al. (1978). This method relies on the ability of vanillin to react with condensed tannin units in the presence of acid to produce a colored complex measured at 500 nm. In this procedure, 200 µl of each extract (at a concentration of 1 mg/ml) were mixed with 3 ml of vanillin reagent (an equal-volume mixture of 8% HCl in methanol and 4% vanillin in methanol). After agitation, the mixture was allowed to react for 20 minutes at 30°C, followed by the measurement of absorbance at 500 nm. Catechin was used as the standard, and the results are expressed in micrograms of catechin equivalents per milligram of extract (µg CE/mg of extract).

Determination of antioxidant activity

The assessment of the trapping capacity of the diphenyl-1-picrylhydrazyl (DPPH) radical was carried out to analyze the antioxidant activity of the methanolic leaf extract of Myrtle, following the protocol by Bouyahya et al. (2016). In this procedure, various concentrations (200, 100, 50, 25, 12.5 µg/ml) of each extract were introduced into test tubes. Subsequently, 2.7 ml of freshly prepared methanolic DPPH solution (0.1 mM DPPH) was added to the prepared extracts. The mixtures were vigorously vortexed and placed in the dark at room temperature for 30 minutes. After incubation, the color change was measured by recording the absorbance at 517 nm.

Antiradical activity of the extracts was expressed as IC50 (concentration resulting in 50% inhibition of free radicals). The mean ic50 values were calculated from linear regressions of three separate trials, where the abscissa represents the concentration of tested extracts, and the ordinate represents the percentage inhibition (PI) of the DPPH radical, calculated using the following formula:

PI % = [(Abs Negative Control– Abs Sample) /Abs Negative Control] X 100

With PI% (Percentage of Antiradical Activity), Abs Sample (Sample Absorbance), and Abs Negative Control

(Absorbance of the negative control, which contains only the DPPH solution). Ascorbic acid was used as a positive control to assess the antioxidant activity of our extracts.

Measurement of leaf morphology

Sampling was conducted using the same batches of leaves collected for the analysis of Myrtle leaf extract profiles and stored at -20°C. Approximately twenty leaves per tree per population were used for the measurement of length (LGFe, cm) and width (LRFe, cm).

Data analysis

A sample number (N) ranging from 9 to 19 trees per population, with a total of 96 trees, is considered for the present study (Table 4). For leaf trait measurements, 20 genotypes per tree per population were considered. For the secondary metabolite compounds studied, 6 genotypes per tree per population were considered.

The obtained data underwent statistical analyses to assess the variability of morphological and phytochemical traits in the methanolic leaf extracts of Myrtle collected from natural populations in Morocco. Descriptive statistics were used to calculate the levels of variation in means by computing the coefficient of variation (CV). To reveal the significance difference between populations a test of LSD was carried. Comparison of means for the studied traits was performed using one-way analysis of variance (Provenance effect). The correlation between morphological and phytochemical traits in the methanolic leaf extracts of Myrtle and environmental factors at the natural sites was assessed based on the Pearson correlation coefficient. All morphological and phytochemical characteristics in the methanolic leaf extracts of Myrtle were used for principal component analysis (PCA) and hierarchical clustering on the matrix of their mean values. Statistical analyses were conducted using SPSS software, version 20.

Conclusion

The present study has allowed us to highlight the potential impact of provenance on morphological traits and the levels of secondary metabolites, such as flavonoids, polyphenols, and condensed tannins, in the leaf extracts of *Myrtus communis*. Additionally, we evaluated the radical-scavenging power of these extracts using the DPPH assay. Based on the results obtained, we conclude that methanolic leaf extracts from Moroccan Myrtle are rich in phenolic compounds, flavonoids, and tannins, and exhibit geographically structured significant antioxidant activity.

Furthermore, the leaf morphological traits, extract yields, and secondary metabolite content of this plant depend on bioclimatic conditions, including temperature, precipitation, and altitude at the sampling sites. Leaf morphological traits and leaf extract yields serve as excellent selection criteria for natural populations, making them valuable for programs aimed at utilizing this species in the food, therapeutic, pharmaceutical, and cosmetic industries.

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