

Comparative Study of Antioxidants in Methanolic Extracts Fruit of Natural Populations of *Myrtus communis* L. in Morocco in Relation to Fruit Size and Environmental Effects

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ABSTRACT

Environmental effects are determining factors for the activity of the antioxidant compounds and fruit form. Samples of *M. communis*, originating from two biogeographically different zones in Morocco, were collected to study the variation in fruit size, fruit extract yield, and content of antioxidant compounds and activity of fruit extract and to examine its association with provenance environmental effect. Analysis of the results shows significant effect of provenance and environmental factors of several traits studied except anthocyanin compounds and IC50. The high content of polyphenols, flavonoids, and extract yield are associated with less watered and warm sites, and with low altitudes. Also, selection for one of the traits of fruit size and/or extract yield could favor selection for one of the phytochemicals. The aggregation of the populations studied using traits studied identify three main groups. These results are interesting for the selection of elite natural populations in favor of programs to promote this species for applications in the food, therapeutic, pharmaceutical and cosmetic fields.

Keywords: antioxidant activity, fruit extract yield, fruit size, *Myrtus communis* L., phenolic compounds, provenance effect.

INTRODUCTION

Myrtle (*Myrtus communis* L., Myrtaceae family) is one of the most used plants in traditional medicine due to its richness in antioxidant compounds beneficial to health (Fadda et al., 2015; Dellaoui et al., 2018; Bouchenak et al., 2020). The ethnobotanical use of Myrtle leaves and berries has been very diverse for several years (Agrimonti et al., 2007; Melito et al., 2016; Aabdousse et al., 2020). It is used in the food industry to flavor, the perfumery and cosmetics industry due to its high content of essential oils from its leaves, flowers and fruit glands, and in traditional medicine as a decoction or infusion to treat several diseases, such as pulmonary disorders, diabetes, hemorrhoids, inflammation and

wound healing (Wahid et al., 2013; Aabdousse et al., 2020). Also, Myrtle is of great importance not only economically but ecologically and socially in the Mediterranean basin (Wahid 2018; Melito et al., 2016; Aabdousse et al., 2021).

Different parts of the plant, leaves, fruits, flowers and roots, find various uses in the food, cosmetics and medicine industries. According to Aabdousse et al. (2020), myrtle leaves are used in Morocco to treat several diseases in a traditional way thanks to its therapeutic virtues. The leaves of this shrub are a crucial source of antioxidants due to their high content of phenolic compounds (Dellaoui et al., 2018; Bouchenak et al., 2020). In general, leaf oils are rich in linoleic acid which is used as a raw material in the manufacture of conjugated linoleic acid (Ma Dwl

et al., 1999). Linoleic acid is a therapeutic nutrient with promising antioxidant and antitumor properties (Belury 2002). On the other hand, the myrtle fruit is very rich in tannins, anthocyanins and other antioxidant compounds (Fadda et al., 2015). These berries are widely used in the industrial formulation of sweet liqueurs (Mulas et al., 2002; Wahid 2013). Thus, the seeds produced as a by-product during the transformation of berries into sweet liquor constitute approximately 36.5% of the entire weight of the berries (Aidi Wannes et al., 2010). Myrtle seed oils are rich in neutral lipids, particularly triacylglycerol, followed by phospholipids and glycolipids (Aidi Wannes and Marzouk 2016). Such as, the total lipids are very rich in linoleic acid. Aidi Wannes and Marzouk (2016) showed that the methanolic extract of myrtle seeds has better antioxidant activity than the seed oil. In recent years, Myrtle has been widely studied for their antioxidant capacity (Aidi Wannes et al., 2010; Fadda et al., 2015; Aidi Wannes and Marzouk 2016; Dellaoui et al., 2018; Bouchenak et al., 2019).

The antioxidant effect of plants is mainly attributed to the phenolic compounds they contain, including flavonoids, phenolic acids and phenolic diterpenes, which have the ability to eliminate free radicals, donate hydrogen atoms or electrons and chelated metal cations. The antioxidant power linked to phenolic compounds exhibits a wide range of physiological properties, including anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial (Pietta et al., 1998; Yanishlieva et al., 2006; Kirka and Arslan 2008). When antioxidants are added to food products, particularly lipid and lipid-containing foods, the capacity and shelf life of food products during processing and storage will be increased. This is by slowing down the lipid peroxidation process which is one of the main reasons for the deterioration of food products (Choe and Min 2006; Yanishlieva et al., 2006). Worldwide, there is

a growing consumer trend towards the use of natural antioxidants in foods. Therefore, supplementing a food product with natural phenols can also have beneficial health effects. There has therefore been great interest in finding and researching the added value of natural antioxidants to replace synthetic antioxidants.

However, the chemical composition of plant extracts could be influenced by environmental conditions, especially pedoclimatic, phenological stages of plants, provenances combined with genetic factors (Ruiz Rodríguez et al., 2014; Maieyes et al., 2015). Depending on these factors, the same species represented by several provenances, populations and genotypes can produce extracts with different antioxidant compound contents. Despite the large number of studies previously carried out on Myrtle, none of them has so far been carried out on the variability of phenolic compounds and antioxidant activity in relation to fruit size in natural populations at Morocco. In this context, the objectives of the present work are (i) define the morphological profile and phenolic compounds of methanolic extracts of fruits collected from natural populations in Morocco, (ii) evaluate their antioxidant activities and (iii) evaluate the relationship between the effect of environmental factors of provenance, the morphological size of the fruits, and between the phenolic composition of the methanolic extracts of the fruits of the natural populations of myrtle in Morocco.

MATERIAL AND METHODS

Plant material

Fruit sampling was carried out systematically between December 2021 and February 2022. Five natural populations of myrtle were collected from different biogeographic regions of

Table 1. Geographic, climatic characteristics (precipitation (Pr), temperature (T)), and number of samples (N) of *Myrtus communis* per population

Populations	Coded	N	Ecological zones	Latitude	Longitude	Altitude (m)	Pr (mm)	T (°C)	Bioclimatic stage
Douar zitouna	IKA	11	Pre-rif	34°43' 53.4"N	04°37' 19.8"W	475	654	17.2	Sub-humide
Izaran	IZA	13	Rif Occidental	34°48' 29.0"N	05°37' 06"W	411	742	18.5	Humide
Aabaïd	BT	11		35°01'48"N	05°09'43"W	745	984	15.9	Humide
Dardara	DAR	11		35°06'11"N	05°16'23"W	450	805	16.9	Humide
Brikcha	BRI			34°55'27.37"N	05°31'45.52"W	252	805	16.90	Sub-humide

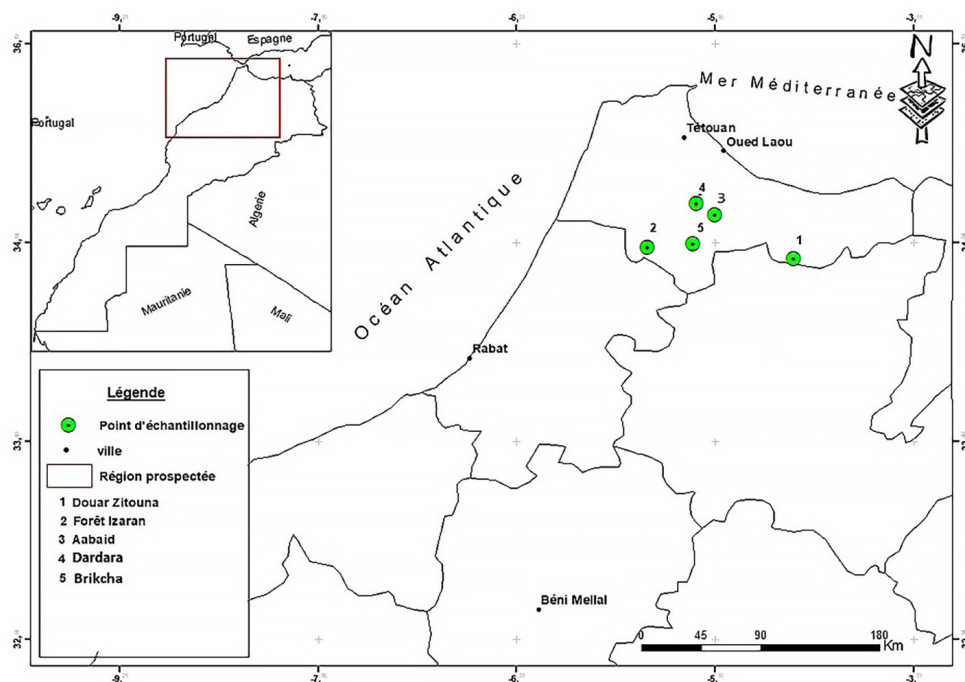


Figure 1. Sampling sites of natural populations of Myrtle studied in Morocco

Morocco (the pre-rif, the western rif, the central plateau and the Middle Atlas) Table 1, Figure 1). For each population, trees were considered randomly for fruit collection. The harvested plant material was dried away from light and humidity, at room temperature. Then, they are stored carefully in paper bags, in a dry place for their use in extract extraction.

Preparation of methanolic extract

The extraction was carried out according to the protocol of Bouyahya et al. (2016). 3 g of myrtle fruits were extracted with 20 ml of pure methanol by maceration at room temperature for 3 days. The crude extract obtained is subjected to filtration by filter paper, then concentrated in a rotary evaporator and finally dried at room temperature. The dry residue recovered is weighed to determine its yield, before storage at 4 °C for subsequent analyses.

Yield calculation

The extraction yield was obtained by the following formula:

$$R(\%) = (Me/Mv) \times 100 \quad (1)$$

where: $R(\%)$ – extraction yield in %; Me – mass of the extract after evaporation of the solvent; Mv – mass of plant material used for extraction.

Assays of total polyphenols

The total polyphenol content of the extracts was determined by the Folin Ciocalteu method (Singleton and Rossi 1965). A volume of 0.5 ml of each extract is added to 2.5 ml of Folin-Ciocalteu reagent (10 times diluted). Then, 2 ml of sodium carbonate Na_2CO_3 (7.5%) is added. The mixture is then incubated in a water bath at 50 °C for 5 minutes. The intensity of the blue coloring produced was measured using a spectrophotometer at a wavelength of 760 nm. The calibration curve was carried out with gallic acid at different concentrations. The results are expressed in milligrams of gallic acid equivalent per gram of dry extract ($\mu\text{g GAE}/\text{mg}$ of extract).

Assay of total flavonoids

The determination of the level of total flavonoids in the methanolic extract of myrtle leaves is carried out by the method described by Tenuta et al. (2002). 250 μl of the extract were added, then 1250 μl of distilled water and 75 μl of 5% sodium nitrite. After 5 min, 150 μl of ammonium trichloride (AlCl_3 2%) is added. After 5 minutes, 500 μl of 1M sodium hydroxide is added, the reaction mixture is made up to 2.5 ml and the solution obtained is vigorously stirred and the absorbance were measured at 510 nm after 30 min. Rutin was

used as a standard to plot the calibration curve. The results were expressed in milligram Rutin equivalent per gram of extract.

Dosage of anthocyanins

Total anthocyanins were determined by the pH variation method described by Kachkoul et al., 2019. 0.5 ml of each extract (1 mg/ml) was added to 3.5 ml of potassium chloride buffer at pH 1.0. The mixture was shaken and incubated for 15 min before measuring the absorbance at 515 and 700 nm. The same procedure was repeated with sodium acetate buffer solution at pH 4.5. The results were expressed in microgram of cyanidin-3-glucoside equivalents per milligram of extract (μg of Cy-3-glcE/mg) and calculated according to the following formula:

$$\text{Anthocyanin content} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L \times C} \quad (2)$$

where: A (absorbance) = $((A_{515} - A_{700}) \text{ pH}_1 (A_{515} - A_{700}) \text{ pH}_{4.5})$; MW – molar mass of cyanidin 3-glucoside (449.2 g/mol); DF – dilution factor; ε – extinction coefficient ($L/(\text{cm} \times \text{mol})$) = 26 900 for cyanidine 3-glucoside; L – optical path (cm); 1000 – gram to milligram conversion factor; C – extract concentration (1 mg/ml).

Total sugars

Extraction of total soluble sugars

Soluble sugars were extracted in accordance with the method of Babu et al. (2002). For this extraction, 0.4 g of sample was crushed with 2 ml (V_{ethanol}) of 80% ethanol subsequently introduced into tubes suitable for centrifugation. That were done at 2000 rpm for 40 min. The supernatant was subsequently introduced into Eppendorf tubes and stored at 4 °C until use.

Dosage of total soluble sugars:

Total soluble sugars were measured using the method of Dubois et al. (1956); 50 μl of extract (V_{extract}) were added to 0.5 ml of 5% phenol and 1.5 ml of sulfuric acid solution (H_2SO_4). The mixture was heated in a water bath at 100 °C for 5 min. After cooling in melting ice, the optical density (OD) was read at 485 nm.

A standard was constructed using a glucose range from a stock solution of 1 mg/mol. The

contents are expressed in mg/g of fresh material. The formula is as follows:

$$\text{Sugar content} = (DO \cdot V_{\text{ethanol}}) / (a \cdot V_{\text{extract}} \cdot PF) \quad (3)$$

where: A – direction coefficient of the calibration line; V_{ethanol} – volume of ethanol in ml; V_{extract} – extract volume in μl ; PF – fresh weight of the plant material used in g; DO – variation of optical density.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test

The antioxidant activity was evaluated by measuring the trapping power of the DPPH radical (1,1-Diphenyl-2-picrylhydrazyl) according to the method described by Bouyahya et al. (2016). 0.3 ml of different concentrations of the methanolic extract is mixed with 2.7 ml of the DPPH solution (0.1 mM). After 30 min of incubation at room temperature and protected from light, the absorbance of fruit extract is measured at 517 nm. The results obtained from measuring the anti-radical activity of DPPH were expressed in relation to those obtained by measuring the activity of Ascorbic acid (vitamin C), the reference antioxidant, at different concentrations in the same conditions. The anti-radical activity of the extracts was expressed as a percentage according to the formula:

$$\%I = (A_{\text{DPPH}} - A_{\text{Sample}}) / A_{\text{DPPH}} \quad (4)$$

where: A_{DPPH} – absorbance of the control (Methanol + DPPH); A_{Sample} – sample absorbance (Extract + DPPH).

The 50% inhibitory concentration (IC50) was determined from the DPPH reduction percentages. The IC50 is expressed in $\mu\text{g}/\text{ml}$ and is compared with that of the standards.

Measurement of fruit morphology

Sampling was carried out at the same batches of fruits collected for the analysis of the profile of Myrtle fruit extracts and stored at -20 °C. Around twenty fruits/tree/population were used to measure length (LGF_e, cm) and width (LRF_e, cm).

Data analysis

The data obtained were the subject of statistical analyzes in order to define the variability of

morphological and phytochemical traits in the methanolic extracts of myrtle fruits collected from natural populations in Morocco. Descriptive statistics made it possible to calculate the levels of variation of the means by calculating the coefficient of variation (CV). The comparison of means for the traits studied was carried out by one-way analysis of variance (Provenance effect). The correlation between the morphological and phytochemical traits in the methanolic extracts of myrtle fruits, and the environmental factors of the natural sites, was carried out based on the Pearson correlation coefficient. All the morphological and phytochemical characters in the methanolic extracts of myrtle fruit were used for a principal component analysis (PCA) and hierarchical classification on the matrix of their average values. Statistical analyzes were carried out by the software (SPSS, version 20).

RESULTS

Variation in fruit size of the populations studied

Table 2 represents the descriptive statistics of the morphological traits of myrtle fruits collected from natural populations. The results show significant variations between populations only in width (LRF_e) (Table 3). In fact, the average fruit length of the populations studied is 10.47 mm. The longest fruits (11.13 mm) are observed in the BRI population of the Western Rif characterized by a subhumid bioclimate. The IKA populations of the subhumid Pre-Rif (10.77 mm) and IZA (10.86 mm) and BT (10.18 mm) of the humid Western Rif have average fruit lengths. The smallest fruit length is reserved for the DAR population (9.38 mm). The same trend of variation in

Table 2. Descriptive statistics of polyphenol compounds, extract yield and morphological traits of natural *Myrtus communis* fruits in Morocco

Populations	Total polyphenols (µg/mg)	Flavonoids (µg/mg)	Sugars (g/100g)	Anthocyanins (µg/mg)	IC50	Yield (%)	LGF (mm)	LRF (mm)	R (LGF/LRF)
IZA	108.71± 16.98	66.55±11.71	1.80±0.25	12.45±3.27	63.67±15.64	9.20±2.28	10.86±0.83 9.44-11.56 7.65	7.69±0.61 6.72-8.26 7.65	1.43±0.03 1.40-1.46 1.92
	95.26-132.50	59.10-87.33	1.47-2.1	8.28-16.43	37.83-77.51	6.67-11.67			
	15.62	17.59	14.06	26.24	24.79	24.79			
IKA	86.28±12.91	58.55±12.68	2.43±0.69	12.99±1.63	60.64±20.74	12.2±1.22	10.77±0.63 10.08-11.79 5.87	8.46±0.32 8.05-8.91 3.83	1.28±0.10 1.18-1.41 7.80
	64.60-98.54	40.27-74.78	1.26-3	10.82-15.10	34.30-79.66	10.67-13.67			
	14.97	21.65	28.27	12.55	34.20	9.96			
BRI	71.14±17.09	44.67 ±7.75	1.42±0.12	14.94±2.98	56.87±23.78	4.27±2.14	11.13±1.05 10.07-12.50 9.44	8.24±0.52 7.58-8.83 6.36	1.37±0.15 1.18-1.54 11.23
	48.55-95.87	38.12-53.61	1.21-1.54	10.55- 18.57	21.67-78.78	2-7.33			
	24.02	17.36	8.70	19.94	41.82	50.15			
DAR	79.36±8.95	37.24 ±2.04	1.74±0.42	14.26±0.88	43.66±7.06	12.17±0.90	9.38±1.45 6.96- 10.60 15.43	6.30±1.20 4.76-7.66 19.02	1.52±0.25 1.28-1.94 16.22
	69.53-92.17	34.20-39.10	1.40-2.42	13.36- 15.50	37.50-55.34	11-13.33			
	11.27	5.48	23.86	6.19	16.17	7.38			
BT	76.98±6.94	49.80 ±7.97	2.01±0.27	13.44±2.04	69.15±5.43	6.67±1.84	10.18±1.15 8.40-11.35 11.34	7.30±0.84 5.82-7.85 11.57	1.40±0.06 1.32-1.47 4.31
	67.07-85.17	41.84-59.29	1.57-2.24	10.95-15.10	61.60-76.05 7.86	4.67 8.67			
	9.02	16	13.50	15.22		27.61			

Table 3. Variance analysis of phenolic compounds, of methanolic extracts yeild of fruits and fruit size of natural populations of Myrtle in Morocco

Traits	Sum of squares	ddl	Average of squares	F	Probability (P)
Yield (%)	241.04	4	60.26	19.66	0.000
Total polyphenols (µg/mg)	4257.35	4	1064.34	6.07	0.001
Flavonoids (µg/mg)	2647.68	4	661.92	7.78	0.001
Sugar (g/100g)	2.94	4	0.73	4.88	0.007
Anthocyan (ug/mg)	20.03	4	5.01	0.93	0.468
IC50	1836.20	4	459.05	1.74	0.181
LGF (mm)	9.85	4	2.46	2.21	0.104
LRF (mm)	14.55	4	3.64	6.28	0.001
R (LGF/LRF)	0.16	4	0.04	2.35	0.089

length between populations is observed in fruit width. Thus, the BRI (8.24 mm) populations of the Western Rif and IKA of the Pre-Rif (8.46 mm) are characterized by larger fruits. Medium fruits are observed in the IZA (7.69 mm) and BT (7.30 mm) population of the humid Western Rif. The smallest fruit width is reserved for the DAR population (6.30 mm). These variations in length and width are reflected in the length versus width ratio of the fruits which showed non-significant variations between the populations studied (Table 3).

Yield of methanolic fruit extract per population

Table 2 shows the results obtained on the yield of fruit extracts by natural population of Myrtle. The one-way analysis of variance (ANOVA) (Table 3) reveals a significant difference between the studied natural populations of Myrtle in yield of methanolic extracts from the fruits. This indicates that the geographical origin of the samples has an impact on the yield. Thus, the IKA populations (12.2%) of the Pre-Rif and DAR of the Occidental Rif (12.17%) present the highest yield of methanolic fruit extract, followed by the IZA population located in Pre-Rif with a value of 9.2%. The BT (6.67%) and BRI (4.27%) populations recorded the lowest fruit extract yields. The variation in the geographical origin of the samples is reflected in the variation in yield between the populations studied.

Analysis of the variance and contents of phytochemical compounds in fruit extracts from natural populations of Myrtle in Morocco

The single-factor analysis of variance (provenance effect) is given in Table 3. We noted a difference between the populations studied that was highly significant at a probability <0.05 , for the contents of polyphenols, flavonoids and sugars. This means that the variations observed in the levels of flavonoids, polyphenols and sugars could be attributed to the geographical origin of the myrtle samples studied. No significant differences between populations were revealed for anthocyanin and IC₅₀ of methanolic fruit extracts.

Polyphenol content

The level of total polyphenols in the fruits of Moroccan common myrtle presents a significant

variation between the populations studied (Table 3). Furthermore, the IZA population of the Western Rif is considered to be the richest in polyphenols with an average level of 108.71 mg EAG/g of extract and of which they vary between 95.26 and 132.50 mg EAG/g of extract with a coefficient of variation of $CV = 15.6\%$. The IKA population of Pre-Rif records an average value of 86.28 ± 12.91 mg EAG/g of extract, with a minimum value of 64.60 and maximum value of 98.54 mg EAG/g of extract ($CV = 15\%$). However, the BRI, DAR and BT populations of the Western Rif have the lowest polyphenol content with an average level of 75.8 mg EAG/g.

Flavonoid content

The flavonoid content in Moroccan *Myrtus communis* fruits varies significantly between populations ($P \leq 0.05$, Table 3). In fact, the IZA population of the Western Rif presents a significant richness in flavonoids with an average content of 66.55 ± 11.71 mg EC/g of extract (Table 2). It varies from 59.10 to 87.33 mg EC/g of extract with a coefficient of variation of 17.6%. The average flavonoid values are reserved for the populations of IKA of the Pre-Rif (58.55 ± 12.68 mg EC/g of extract) and BT of the Western Rif (41.84–59.29 mg EC/g of extract). On the other hand, the BRI (44.67 ± 7.75 mg EC/g of extract) and DAR (37.24 ± 2.04 mg EC/g of extract) populations of the Western Rif record the lowest values of flavonoids (Table 2).

Sugar content

The sugar content of methanolic extracts of Moroccan myrtle fruits also shows a significant variation between the populations studied ($P \leq 0.05$, Table 3). Part of the variation between populations is contributed to the variation within populations (Table 2). However, the Pre-Rif IKA population recorded the highest rate of intra-population variation with $CV = 28.27\%$. On the other hand, the BRI population recorded the lowest intra-population variation with a CV coefficient = 8.7%. In fact, the level of this compound varies from 1.74 ± 0.42 g/100g of extract in the BRI population of the Western Rif as the lowest content to 2.43 ± 0.69 g/100g of extract in the IKA population of Pre-Rif as the richest in sugars (Table 2).

Antioxidant activity of myrtle fruit extracts

The antioxidant activity of the compounds is due to their reducing power. This power is evaluated by the DPPH test. The antioxidant activity of the methanolic extracts of myrtle fruits from five populations was determined by the concentrations which provide 50% inhibition (IC₅₀) compared to the IC₅₀ of a reference free radical scavenger, in this case the ascorbic acid. The IC₅₀ values for the different populations are given by Table 2. However, the one-way analysis of variance does not show a significant variance between the populations studied in terms of antioxidant power (IC₅₀) (Table 3). In fact, the antioxidant activity (IC₅₀) varies from 43.66 ± 7.06 µg/ml for the DAR population of the Western Rif as the highest anti-radical power to 69.15 ± 5.43 µg/ml for the population BT from the Western Rif which presents low antioxidant power (Table 2).

Correlation between phytochemical profile and fruit extract yield, fruit size, and environmental factors

Table 4 shows the correlations between the phytochemical profile and the yield of fruit extracts, fruit size, and environmental factors of the natural sites where this plant thrives. Correlations

between the studied phytochemicals are revealed. Thus, polyphenols have significant positive correlations with flavonoids ($r = 0.83$) and negative correlations with anthocyanins ($r = -0.83$). Also, polyphenols are moderately correlated with extract yield ($r = 0.37$). Flavonoids are significantly correlated with anthocyanins ($r = -0.88$) and IC₅₀ ($r = 0.68$), fruit size (LGF: $r = 0.64$; LRF: $r = 0.58$), and moderately correlated with sugars ($r = 0.47$). Average correlations are revealed between sugars and anthocyanins ($r = -0.69$) and extract yield ($r = 0.6$). The yield of methanolic extract from fruits shows a negative correlation with anthocyanins ($r = -0.41$) and with IC₅₀ ($r = 0.43$). Fruit size is correlated with IC₅₀ (LGF: $r = 0.56$; LRF: $r = 0.53$), and with the yield of fruit extracts (LGF: $r = -0.48$). Knowing that fruit size and extract yield are easily measured. Selection according to one of the traits of fruit size and/or extract yield will be in favor of phytochemicals selection.

Depending on the environmental factors studied at the site, it can be said that the expression of each of the secondary metabolite compounds studied takes place under site-specific environmental stress conditions. Furthermore, precipitation has a moderate negative effect on polyphenols ($r = -0.48$), flavonoids ($r = -0.56$), sugars ($r = -0.34$), yield ($r = -0.4$) and fruit size (rLRF = -0.57 ; rLGF = -0.65), and positive with anthocyanins (r

Table 4. Phenotypic correlation between phytochemical contents, morphological traits and environmental factors of Myrtle fruit in Morocco

Traits	Total polyphenol (µg/mg)	Flavonoids (µg/mg)	Sugars (g/100g)	Anthocyanins (µg/mg)	IC ₅₀	Yield (%)	LGF (mm)	LRF (mm)	Precipitation (mm)	Temperature (°C)	Altitude (m)
Total polyphenols (µg/mg)	1										
Flavonoids (µg/mg)	0.825	1									
Sugars (g/100g)	0.252	0.472	1								
Anthocyanins (µg/mg)	-0.831	-0.877	-0.689	1							
IC ₅₀	0.262	0.676	0.381	-0.579	1						
Yield (%)	0.369	0.097	0.592	-0.412	-0.427	1					
LGF (mm)	0.249	0.638	-0.004	-0.204	0.561	-0.483	1				
LRF (mm)	0.097	0.578	0.289	-0.215	0.530	-0.285	0.922	1			
Precipitation (mm)	-0.475	-0.563	-0.339	0.347	0.059	-0.400	-0.566	-0.652	1		
Temperature (°C)	0.760	0.376	-0.141	-0.324	-0.383	0.471	0.069	-0.094	-0.585	1	
Altitude (m)	-0.231	0.189	0.333	-0.227	0.838	-0.482	0.187	0.249	0.471	-0.802	1

= 0.38). Temperature presents strong positive correlations with polyphenols ($r = 0.76$), followed by moderate correlations with flavonoids ($r = 0.38$), yield ($r = 0.47$), anthocyanins ($r = -0.32$), and the IC50 ($r = -0.33$). Altitude has an effect on IC50 ($r = 0.84$) and performance ($r = -0.48$). These characteristic compounds of the methanolic extract of myrtle fruits in Morocco are clinal with precipitation, temperature and altitude. This means that high levels of polyphenols, flavonoids, and yield may be associated with less watered and warm sites, and at lower altitudes.

Geographic structure of Myrtle populations in relation to the traits studied

Principal component analysis (PCA) using the mean values of the studied variables shows that 72% of the total variation between the studied populations was explained by the first two components (Figure 2). The first component (PC 1) explains 43.15% of the total variation linked to the content of polyphenols, flavonoids, yield, and fruit size. The second component (PC 2) explains 28.85% of the total variation associated with the anthocyanin content, the IC50 of the extracts, and the sugar levels. Figure 2 visualizes the projection of the populations studied in relation

to the projection of the first axes. Analysis of the results in Figure 2 shows the separation of the populations into three groups. We note the presence of resemblance between the populations of different provenances (Pre-Rif and Western Rif) and the dissimilarity between populations of the same provenance (Western Rif) having the same biogeographic conditions. Group 1 (G1) is represented by the IKA populations of the Pre-Rif and IZA of the Western Rif, characterized by a different subhumid and humid bioclimate respectively. These IKA (475 m) and IZA (411 m) populations are distributed over close altitudes (Table 1). The second group (G2) brings together the populations of the Western Rif BRI and BT. This populations have different bioclimatic stages (Subhumid and humid respectively) with high precipitation amounts ranging from 984 (BT) and 805 mm (BRI) (Table 1). Also, they are characterized by different altitudes 745 (BT) and 252 m (BRI). The third group (G3) is represented by a DAR population from the Western Rif. It is characterized by a different bioclimate (humid and subhumid, respectively) and close precipitation ranging from 601 (IZA) to 753 (BT) mm, Middle Atlas (BE, semi-arid, 389 mm), and Central Plateau (OULK, subhumid, 475 mm). The hierarchical classification by the aggregation method of the populations

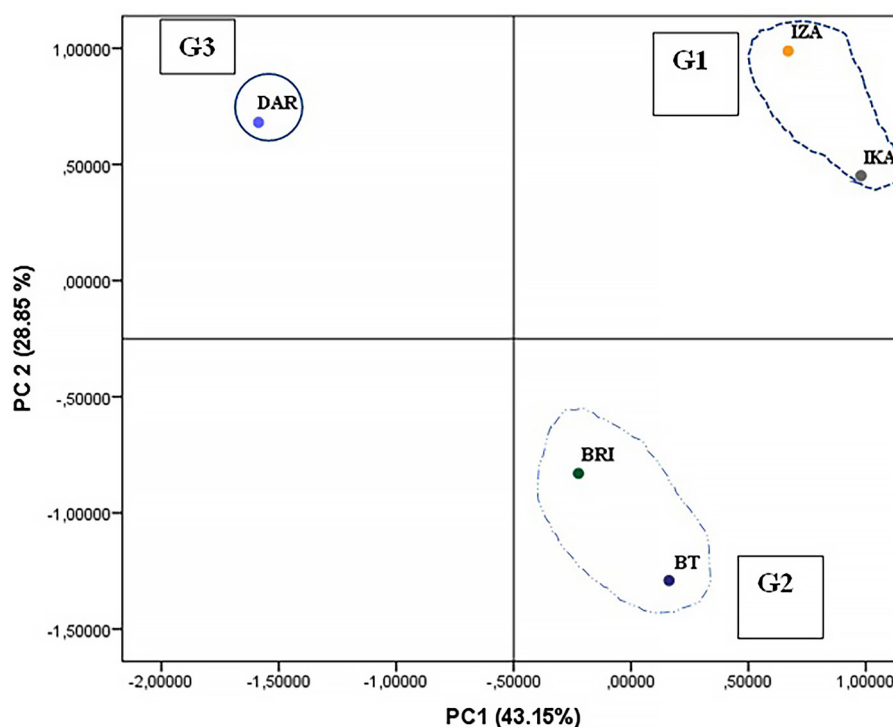


Figure 2. Principal component analysis of the variability in the yield of methanolic fruit extracts, phytochemical compounds studied, fruit size and environmental factors at the common myrtle site in Morocco

studied is presented in Figure 3. The analysis of Figure 3 allowed the identification of three main groups (D1, D2 and D3). The first group (D1) is made up of three populations IZA and BRI from the Western Rif, and IKA from the Pre-Rif. The second group (D2) is represented by a single DAR population. The third group (D3) is made up of the BT population.

DISCUSSION

Fruit size, content of antioxidant's studied and effect of population

The size of Myrtle fruits is easy to measure and is a good indicator for genetic selection in favor of individuals richest in phenolic compounds. High variation between and within the population for fruit width is shown in the present study. The difference in the genetic potential of Myrtle populations in Morocco based on fruit morphological traits is well demonstrated by other authors (Wahid et al., 2018; Abdousse et al., 2021). This variability observed within the population in terms of fruit size will promote the adaptability of *Myrtus communis* L. in the face of climate change. The variability at the Myrtle fruit level would allow a diversity of richness in phenolic compounds.

The yield of methanolic extracts from myrtle fruits varies remarkably between the populations

studied. This shows that the provenance of the Myrtle plant material has a significant effect on the yield of fruit extracts (Fadil et al., 2016; Yanguia et al., 2021). Indeed, the IKA populations (12.2%) of the Pre-Rif and DAR of the Western Rif (12.17%) present the highest yield of methanolic fruit extract. The variability observed in the yield of the extracts could be due to the polarity of the solvent used (Athamena et al., 2019) and also to intrinsic factors linked to the specimens, namely: genetics, the vegetative stage and the collection period, the cycle vegetative, etc. (Yanguia et al., 2017; 2021). Also, the variability observed both in the yield of methanolic extracts and in chemical compounds between the areas studied could be explained by factors in the environment of the sites, such as climate, eco-climatic, edaphic factors, exposure, orography, altitude, etc. (Fadil et al., 2016). In addition, the adaptive power of the plant in the face of these factors could influence the preferential biosynthesis of chemical compounds and their content, which results in their differential expression.

The analysis of the contents of phytochemical secondary metabolites of fruit extracts, in particular those related to polyphenols, flavonoids and sugars, demonstrated variability between natural populations of Myrtle in Morocco. Anthocyanins and IC50 do not show variation between populations but rather intra-population variation which is mainly attributed to genetic factors.

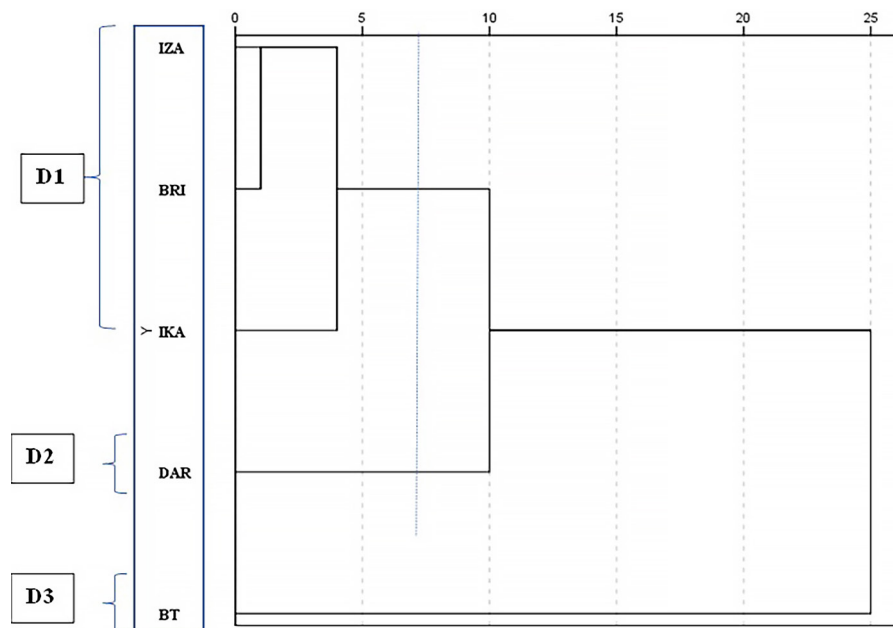


Figure 3. Hierarchical classification of natural populations of Myrtle in Morocco using Euclidean distance based on the variables studied

The variability between populations could be explained by the difference in environmental and genetic expression of the populations collected from the Pre-Rif and Western Rif. Differences in environmental conditions, such as precipitation, temperature, orography, soil type, which characterize distinct biogeographic zones, could explain the provenance and population effect. Several authors, using the same traits studied and/or others have confirmed the difference between natural populations or genotypes (individual variation) of Myrtle (Messaouda et al., 2007; Fadil et al., 2016; Yanguia et al., 2021). So we show that provenance could be the origin of variability between populations. The provenance by these characteristics, in particular, geographical, edaphic and climatic could be the origin of the variation in the contents of secondary metabolites and consequently the biological potential of the plant extracts.

Furthermore, the polyphenol and flavonoid contents of methanolic extracts of Myrtle fruits in Morocco are high compared to other national regions. For example, Amensour et al. (2009) recorded polyphenol contents in bulk chefchaoun fruit extracts 14.7 mg GAE/g of extract. On the other hand, work on Tunisian myrtle has also shown higher polyphenol values compared to those encountered on Moroccan myrtle (Yanguia et al., 2021). However, the content of phenolic compounds or other secondary metabolites could depend on the polarity of the solvent used, the part of the plant subject to extraction, the condition of the plant material and the extraction method used (Athamena et al., 2019). The richness in polyphenol and flavonoids reveals the presence of specific bioactive components in myrtle fruits which could be responsible for the antioxidant activity. The variation in the richness of polyphenols and flavonoids in natural populations would be a potential choice for elite individuals in the antioxidant activity of botanical extracts. The use of synthetic antioxidants could be avoided by replacing them with natural antioxidants rich in antioxidant activities.

The sugar in methanolic extracts of Moroccan Myrtle fruits is under the effect of provenance linked to genetic and environmental effects. Aouadi et al. (1991) noted that the quantity of polysaccharide substances in the plant varies greatly depending on its origin, the variety and also the stage of growth. This result shows the fruit presents a different energy richness linked to sugar between populations. It would be necessary

to take into consideration the selection of elite genotypes to improve sugar yield as being energetic food elements necessary for the function of biological and structural activities of the cell, and also for industrial applications. It is noted that the polysaccharide fraction isolated from plant fruits can be considered as a potential candidate for the development of a new anti-diabetic agent, and to neutralize free radicals (Ying-Kun et al., 2008). In recent years, some bioactive sugars isolated from natural sources have attracted much attention in the field of biochemistry and pharmacology. They exhibit various biological activities. A study was reported by Aouadi et al. (1991) which show the antitumor activity of extracellular sugar from Basidiomycete. Several studies on plant polysaccharides in general reveal that they have strong antioxidant activities and can be explored as potential new antioxidants (Ying-Kun et al., 2008; Ge et al., 2009; Chidouh 2014).

Correlation of studied traits

Correlations between the studied phytochemicals are revealed. Thus, polyphenols are significantly and positively correlated with flavonoids ($r = 0.83$). This indicates that flavonoids constitute the dominant phenolic group in Myrtle fruits. Shahat et al. (2016) showed that flavones constitute the main class of flavonoids present in fruits. In general, less polar flavonoids can exist at high concentrations and therefore contribute to important biological activities (Stankovic et al. 2011). Flavonoids are considered a subclass of phenolic compounds, it is therefore logical that the total polyphenol content of the extracts is directly related to their flavonoid content. Consequently, the factors acting on the polyphenol content necessarily influence the composition of flavonoids. The two compounds phenols ($r = -0.83$) and flavonoids ($r = -0.88$) as well as sugars ($r = -0.69$) are strongly and negatively correlated with anthocyanins. The richer the sample is in phenol, flavonoid and sugar, the less concentrated it is with anthocyanins. This result could be explained by the nature and polarity of the extraction solvent. Furthermore, nonpolar antioxidants can show greater antioxidant activities in emulsions since they concentrate at the lipid phase, unlike polar antioxidants which remain in the aqueous phase and therefore exhibit low activities.

Flavonoids are correlated with the IC₅₀ ($r = 0.68$). This reflects that *M. communis* fruit extract

rich with flavonoid content will exhibit higher DPPH inhibitory activity. Obviously, the relationship of DPPH scavenging and flavonoid activity is justified by their ability to destroy radicals due to their hydroxyl groups. Indeed, several previous studies have demonstrated a strong correlation between the reducing capacity of extracts and the presence of antioxidant compounds (Lesjak et al., 2011; Taviano et al., 2013; Keskes et al., 2014). Furthermore, polyphenolic or flavonoid compounds are used as traps for peroxide radicals and intermediate alkoxy radicals, as well as chelating agents for metal ions, which play an essential role in the initiation step of radical reactions. Subsequently, we note that the content of the antioxidant activity of the extracts from the areas studied could be explained by their richness in flavonoid compounds. For this reason, we observed that populations rich in flavonoids exhibit significant reducing power. Thus, the relationship between antioxidant compounds shown in the present study is good indicative of whether the selection of one compound will contribute to the selection of the other. However, it would be better to look for the relationship of these antioxidant compounds with easily measurable yield or fruit size.

The yield of methanolic extract from fruits shows a moderate correlation with phenols ($r = 0.37$), sugars ($r = 0.6$) and with the IC50 ($r = 0.43$). Also, fruit size is correlated with flavonoids (LGF: $r = 0.64$; LRF: $r = 0.58$), IC50 (LGF: $r = 0.56$; LRF: $r = 0.53$), and with the yield of fruit extracts (LGF: $r = -0.48$). Therefore selection according to one of the traits of fruit size and/or extract yield could be in favor of the selection of one of these phytochemicals (Phenols, flavonoids, sugars and IC50).

Depending on the environmental factors studied at the site, it can be said that the expression of each of the secondary metabolite compounds studied takes place under site-specific environmental stress conditions. In the present study, a clinal relationship was shown between the antioxidant compounds characteristic of the methanolic extract of myrtle fruits in Morocco and with precipitation, temperature and altitude. Furthermore, precipitation has a moderate and negative effect on polyphenols ($r = -0.48$), flavonoids ($r = -0.56$), sugars ($r = -0.34$), yield ($r = -0.4$) and fruit size (rLRF = -0.57 ; rLGF = -0.65). Temperature presents strong positive correlations with polyphenols ($r = 0.76$), flavonoids ($r = 0.38$), and yield ($r = 0.47$). Altitude has an effect on the IC50 ($r =$

0.84) and the yield of methanolic fruit extract ($r = -0.48$). Environmental factors are determining factors for the presence or absence of the antioxidant compounds studied in the methanolic extract of *M. communis* fruit. This means that high levels of polyphenols, flavonoids, and yield may be associated with less watered and warm sites, and at lower altitudes.

Geographic structure of natural populations of Myrtle

The aggregation of the populations studied using fruit size, extract yield and content of antioxidant compounds studied made it possible to identify three main groups. The first group (D1) is made up of three populations IZA and BRI from the Western Rif, and IKA from the Pre-Rif. This D1 group is characterized by a similarity in the values of most of the characteristics studied, in particular by high contents of polyphenols and flavonoids in methanolic fruit extracts and by large fruit size. Also, the D1 populations are characterized by average yields of methanolic fruit extracts and average contents of anthocyanins, sugars and IC50. The second group (D2) is represented by a single DAR population. This population is characterized by moderate values of total polyphenols and sugars. The yield of methanolic DAR extracts is high but the fruit size is low. The third group (D3) is made up of the BT population. It is characterized by moderate contents of total polyphenols and flavonoids and by an average fruit size. The BT population presents high contents of anthocyanin, sugars, and IC50.

CONCLUSION

This study highlighted the variation in size, extract yield and content of antioxidant compositions and activity of *M. communis* fruit extracts from different populations in Morocco. Such variation is mainly attributed to genetic factors but to environmental factors. The provenance and environmental effects are well noted by the present study. Environmental factors are determining factors for the presence or absence of the antioxidant compounds studied in the methanolic extract of *M. communis* fruit. This means that high levels of polyphenols, flavonoids, and yield may be associated with less watered and warm sites, and at lower altitudes. Also, selection according to one

of the traits of fruit size and/or extract yield could be in favor of the selection of one of the phytochemicals (phenols, flavonoids, sugars and IC50). The aggregation of the populations studied using fruit size, extract yield and content of antioxidant compounds studied made it possible to identify three main groups. Future studies on the current topic are recommended in order to select the most bioactive compound which could serve as a basis for future application of Myrtle in food, therapeutic, pharmaceutical and cosmetic industries.

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