Trends Phytochem. Res. 3(4) 2019 231-242

Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

Original Research Article

Morphological and chemical characterization of two wild Tunisian myrtle (*Myrtus communis* L.) populations

W. Aidi Wannes[1*], M. Saidani Tounsi And B. Marzouk[1]

[1]Laboratory of Aromatic and Medicinal Plants, Biotechnologic Center of Borj-Cedria, BP 901, 2050 Hammam-Lif, Tunisia

ABSTRACT

Myrtle is an aromatic and medicinal shrub growing wild in the Mediterranean regions. The objective of this study was to determine qualitative and quantitative morphological characteristics and to analyze the biochemical composition of two wild Tunisian myrtle populations from Bizerte (BM) and Haouaria (HM). These two populations presented the same vegetative characters but with some morphological differences such as the bigger size of BM fruits and leaves. The biochemical characterization revealed that there was an increase in the production of phenols during flowering in both populations, and there was a significant variation in their levels in the various organs. The phenolic fraction of myrtle leaf and the fruit was rich in tannins while the stem was rich in flavonoids. Methanol extracts of different myrtle parts, especially leaf, presented strong antioxidant activities.

ARTICLE HISTORY

Received: 19 June 2019
Revised: 23 July 2019
Accepted: 15 November 2019
ePublished: 07 December 2019

KEYWORDS

Antioxidant activity
Biochemical composition
Morphological characters
Myrtus communis

1. Introduction

The Myrtaceae is a large family of dicotyledonous woody plants placed within the order Myrtales containing over 3000 species organized in 100 genera (Marchiori and Sobral, 1997). Myrtle (*Myrtus communis* L.), belonging to the Myrtaceae family, is a shrub native to the Mediterranean countries and western Asia pushing mainly in scrubland and maquis. Ancient Mediterranean populations have largely used myrtle for its ornamental and aromatic values (Agrimonti et al., 2007). Myrtle fruits have a long history of application as food replacing pepper as a spice for meat cooking and as food integrators because of its high vitamin contents (Marcini and Maccioni, 1998). At the folk medicine, fruit decoction or infusion of this plant was used as stomachic, hypoglycemic, cough and oral diseases, anti-microbic, for constipation, appetizing, anti-hemorrhagic and externally for wound healing (Özek et al., 2000). At present, the greater part of myrtle produced in the Mediterranean area are used for liqueur preparation (Mulas, 2012). Recently, this plant has been appreciated by food, pharmaceutic and cosmetic industries owing to its nutraceutical and antioxidant properties (Barboni et al., 2010; Sanna et al., 2019). Much researches have been reported on the essential oil composition of *M. communis* (Messaoud et al., 2005; Tuberoso et al., 2006; Aidi Wannes et al., 2007; Aidi Wannes et al., 2009; Aidi Wannes and Marzouk, 2012; Usai et al., 2015; Scazzocchio et al., 2016; Anwar et al., 2017; Tardugno et al., 2018; Usai et al., 2018). More recently, the pharmacological proprieties of this essential oil have been deeply explored. Anti-microbial (Cannas et al., 2013; Aleksic and Knezevic, 2014; Scazzocchio et al., 2016; Anwar et al., 2017, Tardugno et al., 2018; Khan et al., 2019). anti-hyperglycemic (Panjeshahin et al., 2016; Talebianpoor et al., 2019) and antioxidant (Tuberoso et al. 2007; Aidi Wannes et al. 2010; Aidi Wannes and Marzouk, 2013; Anwar et al., 2017; Aidi Wannes and Marzouk, 2016). activities have been also reported. A part essential oil, several studies have also valorized this plant through its fatty acids (Aidi Wannes et al. 2009; Serce et al., 2010; Aidi Wannes and Marzouk, 2011; Aidi Wannes and Marzouk, 2016) and phenolics (Aidi Wannes et al., 2010, Aidi Wannes and Marzouk, 2013; Aidi Wannes and Marzouk, 2016). In Tunisia, myrtle grows under *Quercus suber* L. and *Quercus faginea* Lamk. forests in humid and subhumid bioclimatic stages. However, in Cap Bon and Tunisian dorsal, myrtle is found in association with *Pinus*

✉Corresponding author: Wissem Aidi Wannes
Tel: +21697547029; Fax: +21679412638
E-mail address: aidiwanneswissem18@gmail.com

halepensis Mill, *Juniperus phoenicea* L., *Ceratonea siliqua* L. and with shrubs such as *Pistacia lentiscus* L. (Messaoud et al., 2005). Most of the myrtle biomass has been harvested from wild plants without consideration of the reduction of natural biodiversity (Melito et al., 2016). Subsequently, the natural populations are progressively decreasing in number and size (Messaoud et al., 2006). Additionally, the phytochemical production depends upon internal and external factors affecting the plant as genetic structures and environmental conditions. In fact, the investigation of the chemical and morphological diversity of myrtle populations represented an essential step to verify the biomass production, and to constitute an important resource for agro-industrial purposes. So, the aim of this work was the detection of morphological and biochemical differences between two Tunisian myrtle populations.

2. Experimental

2.1. Plant material

From Fig. 1, myrtle populations were collected from Jebal Stara in Haouaria region (HM population; North East of Tunisia-Nabeul; latitude de 37°3'0.13''N; longitude 11°2'43.48''E; altitude 141.43 m) and from Jebal Er-Rimel in Menzel Jemil region (BM population; North East of Tunisia-Bizerte; latitude 37°15'39.13''N, longitude 9°53'49.58''E; altitude 150 m). Botanical identification of this species was carried out by Prof. A. Smaoui (Biotechnologic Center in Borj-Cedria Technopark, Tunisia) according to the Tunisian flora (Pottier-Alapetite, 1979). A voucher specimen has been kept in our unit for future reference. The aerial parts of the two myrtle populations (BM and HM) were collected during a whole year throughout their vegetative cycle from two different regions belonging to the subhumid stage. Additionally, these two stations were characterized by calcareous soil.

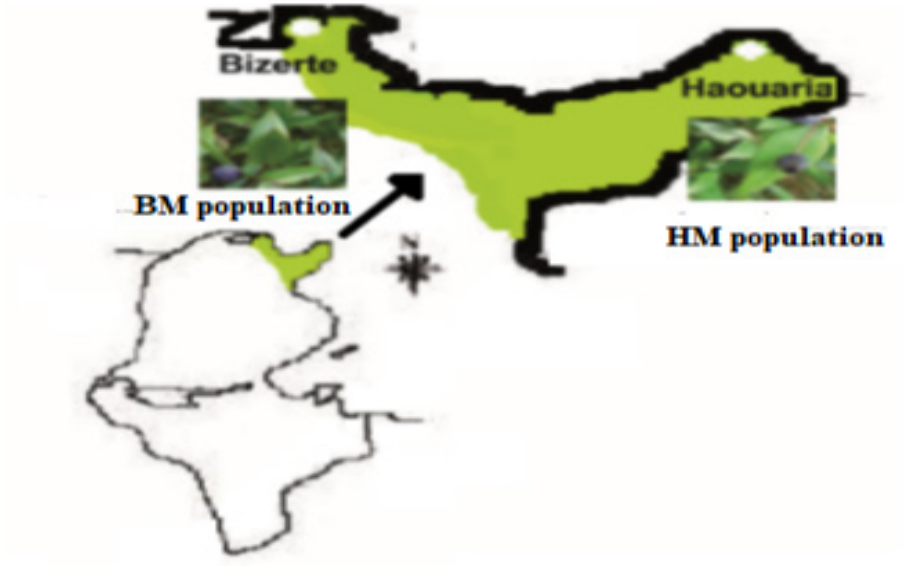

Fig. 1. Localisation of sample areas (Bizerte and Houaria)

The meteorological data was taken from the Tunisian Meteorological Station (Table 1). The aerial parts of the two myrtle populations (BM and HM) were collected during a whole year throughout their vegetative cycle from two different regions belonging to the subhumid stage. Additionally, these two stations were characterized by calcareous soil. The meteorological data was taken from the Tunisian Meteorological Station (Table 1). Myrtle leaves, fruits and stems were isolated manually from the aerial parts in our laboratory to obtain a weight of 500 g of each part. These parts were air-dried and stored in the dark at ambient temperature until use for further analysis.

2.2. Morphological analysis

Measurements of the longitudinal and lateral growth of shrubs of two populations were carried out directly on the plants in the natural environment. Morphological characterization of leaves and fruits by determining their dimensions (width and length) was performed on dry samples. For this, ten samples each containing ten plants are taken. After each sample, different myrtle parts (leaf, fruit, stem) of both populations (BM and HM) were used to determine the fresh weight (FW) and the dry weight (DW). These samples were heated in an oven at 100 °C. to constant weight. The fresh and dry weight measurements were repeated three times and the final result is the average of these three measurements affected by the standard error.

2.3. Chemical analysis

2.3.1. Preparation of plant extract

This extraction was done according to a method inspired by that of (Mau et al. 2001). by mixing 1 g of

dry vegetable material with 10 mL of methanol. The mixture was kept at rest for 24 hours at 4 °C in the dark. Finally, this mixture was filtered on ashless filter paper (Watman No. 4). The extracts thus obtained were stored at 4 °C. for the various subsequent analyzes.

2.3.2. Total polyphenol content

The determination of total polyphenols (PT) was carried out by spectrophotometry (Dewanto et al., 2002). The polyphenol contents are expressed in mg of gallic acid equivalent per gram of dry matter (mg EAG / g DW).

2.3.3. Total tannin content

Table 1
Meteorological data of myrtle sampling during vegetative cycle from Bizerte and Haouaria.

	Bizerte myrtle population (BM)		Haouaria myrtle population (HM)	
Sampling date (DAF)	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
February (210 DAF)	11.4	82.9	12.1	16.5
March (240 DAF)	13.7	44.7	14.4	46.5
April (270 DAF)	17.7	9	18.1	18.9
May (300 DAF)	20.8	28.2	21.4	48.9
June (330 DAF)	24.1	7.1	23.8	7.8
July (0 DAF)	26.7	0	27.9	19.8
August (30 DAF)	26.3	1.6	27.4	27.1
September (60 DAF)	23.7	70.8	24.1	83.4
October (90 DAF)	22.0	40.2	22.6	123.2
November (120 DAF)	16.8	55.6	17.9	15.6
December (150 DAF)	13.2	156.8	14.7	132.8
January (180 DAF)	12.6	26.5	14	55.9

DAF: Days after flowering

The determination of total tannins (TT) is determined by the Folin-Ciocalteu reagent as described by (Hagerman and Butler 1978). TT contents are expressed as is the case for PT in mg GAE/g DW.

2.3.4. Total condensed tannin content

The condensed tannins (T) were measured by spectrophotometry (Sun et al., 1998). The contents of condensed tannins are expressed in mg CE/g DW.

2.3.5. Total flavonoid content

Total flavonoids (TF) were assayed by a colorimetric method according to (Dewanto et al. 2002). The flavonoid contents were expressed as for T in mg of catechin equivalent per gram of dry matter (mg CE/g DW).

2.3.6. DPPH activity

The estimate of this antiradical activity is measured according to the method of (Hanato et al. 1988). 1 mL of different extract concentrations (1, 10, 100 and 200 µg/mL) prepared in methanol were added to 0.5 mL of a 0.2 mmol/L DPPH methanol solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. BHT was used as a positive control.

2.3.7. Reducing power

The method of (Oyaizu 1986). was used to measure the reducing power of different organ extracts. 1 mL of different extract concentrations (1, 10, 100 and 200 µg/mL) prepared in methanol were mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6, prepared from 62.5 mL of a 0.2 M Na2HPO4 and 37.5 mL of 0.2 M NaH2PO4H2O) and 2.5 mL of 1% K3Fe(CN)6 and incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added to the mixture that was centrifuged at 650g for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the blue-green colour was measured at 700 nm. Ascorbic acid was used as a positive control.

2.3.8. β-Carotene bleaching assay

The monitoring of the writing kinetics is done by spectrophotometry according to the method described by (Koleva et al. 2002). β-Carotene (2 mg) was dissolved in 20 mL chloroform and to 4 mL of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40 °C and 100 mL of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. Reference compounds (BHT and BHA), sample extracts were prepared in methanol. The emulsion (3 mL) was added to a tube containing 0.2 mL of different extract concentrations (1, 10, 100 and 200 µg/mL). The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50

°C for 120 min, when the absorbance was measured again. BHT and BHA were used as positive control.

2.4. Statistical analysis

All the analyzes are done in three repetitions. Data are expressed as mean ± SD and the comparison of the averages was done by Analysis of the Variance (ANOVA).

3. Results and Discussion

3.1. Morphological characterization

3.1.1. Dimensional growth

In order to characterize the two myrtle populations from Bizerte (BM) and Houaria (HM), a comparison of the dimensional growth (length and width) of *M. communis* aerial parts was carried out (Table 2).

3.1.1.1. Myrtle shoot

BM had the highest length and width reaching 1.47 and 1.14 m respectively at the flowering period while HM had only 0.87 m of length and 0.77 m of width at this stage of development. In addition, both populations reacted in the same way during their vegetative cycle. First there was a progressive increase in longitudinal and lateral growth of plant during the vegetative period (from February to June) until reaching a maximum at flowering. Beyond this stage, growth became slow and this could be due to the fact that the plants were in the fruiting period. Regardless the development stage, the shrubs of BM were more developed than those of HM. However, these two myrtle populations produced much longer shoot than those studied by (Mulas and Cani 1999). in the case of 70 wild Sardinian myrtle ecotypes with shoot length did not exceeding 0.19 m.

3.1.1.2. Myrtle leaf

The length and width of myrtle leaf in both populations of myrtle were also determined (Table 2). The dimensional difference was significant between the two myrtle leaves. The average length of HM leaves was 38.99 mm and 36.81 mm for BM. As for the average

Table 2
Morphological characterization of two myrtle populations from Bizerte (BM) and Haouaria (HM)

	Population	February	March	April	May	June	July
Shrub length (m)	Bizerte	1.17±0.17	1.20±0.02	1.19±0.02	1.21±0.02	1.10±0.18	1.47±0.06
	Haouaria	0.69±0.16	0.65±0.11	0.60±0.06	0.72±0.07	0.79±0.20	0.87±0.04
Shrub width (m)	Bizerte	0.75±0.16	0.76±0.11	0.80±0.12	0.81±0.06	0.90±0.11	1.14±0.10
	Haouaria	0.60±0.06	0.63±0.11	0.59±0.07	0.61±0.07	0.64±0.10	0.77±0.11
Leaf length (mm)	Bizerte	38.50±0.47	38.66±0.62	38.65±0.65	38.75±0.08	38.78±0.12	38.99±0.38
	Haouaria	36.40±0.21	36.41±0.0.20	36.55±0.20	36.56±0.02	36.71±0.15	36.81±0.42
Leaf width (mm)	Bizerte	18.40±0.13	18.49±0.20	18.50±0.15	18.66±0.20	18.70±0.04	18.75±0.17
	Haouaria	11.30±0.16	11.38±0.16	11.44±0.05	11.47±0.19	11.49±0.21	11.54±0.22
Fruit length (mm)	Bizerte	-	-	-	-	-	-
	Haouaria	-	-	-	-	-	-
Fruit width (mm)	Bizerte	-	-	-	-	-	-
	Haouaria	-	-	-	-	-	-

	Population	August	September	October	November	December	January
Shrub length (m)	Bizerte	1.42±0.04	1.30±0.07	1.40±0.20	1.20±0.02	1.40±0.20	1.35±0.10
	Haouaria	0.86±0.02	0.80±0.11	0.85±0.15	0.82±0.30	0.83±0.10	0.82±0.30
Shrub width (m)	Bizerte	1.01±0.28	0.95±0.14	1.01±0.27	0.95±0.14	0.85±0.10	0.90±0.11
	Haouaria	0.64±0.10	0.62±0.06	0.58±0.08	0.58±0.08	0.59±0.07	0.60±0.06
Leaf length (mm)	Bizerte	38.87±0.05	38.86±0.05	38.85±0.05	38.87±0.07	38.88±0.57	38.83±0.30
	Haouaria	36.80±0.76	36.79±0.45	36.71±0.14	36.73±0.05	36.73±0.07	36.71±0.15
Leaf width (mm)	Bizerte	18.73±0.55	18.72±0.56	18.71±0.04	18.70±0.04	18.71±0.04	18.65±0.17
	Haouaria	11.53±0.03	11.53±0.03	11.52±0.03	11.51±0.02	11.50±0.14	11.48±0.20
Fruit length (mm)	Bizerte	4.51±0.03	5.00±0.22	6.72±0.15	9.10±1.05	11.33±0.03	11.70±0.19
	Haouaria	5.11±0.55	4.90±0.25	5.90±0.73	9.80±0.28	11.30±0.08	11.40±0.22
Fruit width (mm)	Bizerte	4.10±0.27	4.50±0.43	5.50±0.37	7.90±0.73	8.20±0.78	8.30±0.91
	Haouaria	2.20±0.32	3.30±0.95	4.10±0.46	6.20±0.30	7.20±0.59	7.40±1.23

Values given are the means of three replicates ± standard deviation.

width of the leaves, it was 18.75 mm for BM and 11.54 mm for HM. These results were comparable to those of (Pottier-Alapetite 1979) who indicated that BM leaves were larger than those of HM. Similar results were also obtained by (Mulas and Cani 1999) who determined the leaf lengths (15-44 mm) and widths (8-24 mm) of 70 wild myrtle ecotypes from Sardinia. In this study, myrtle leaves were small in both populations during the vegetative period which could be due to an intense vegetative propagation at this stage and the formation

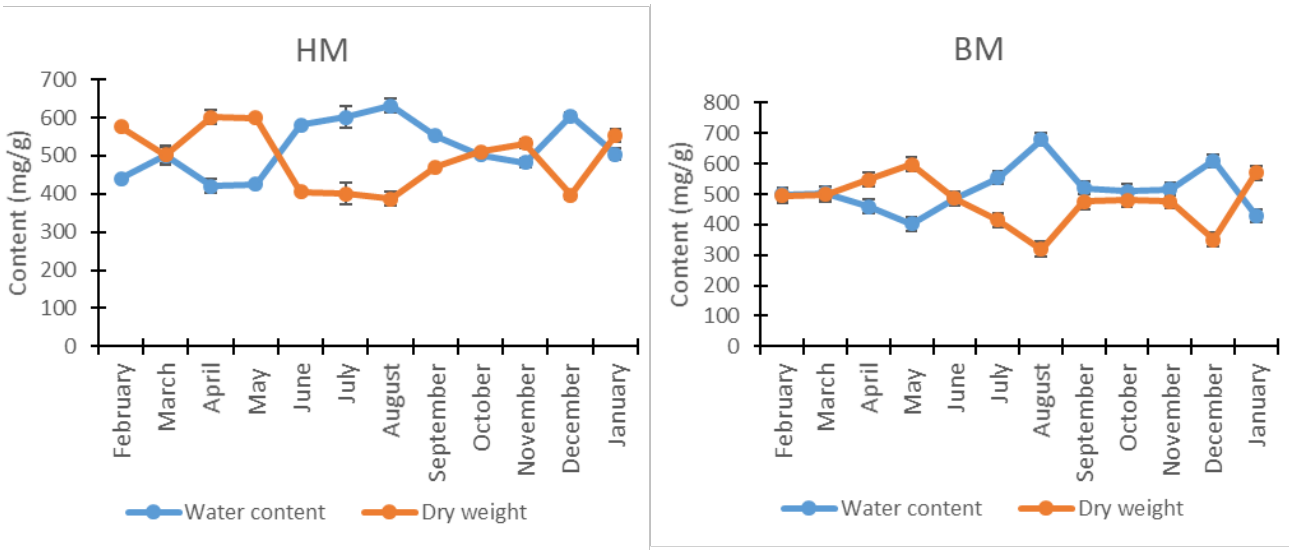

Fig. 2. Evolution of dry weight and water content of myrtle leaf during development of two myrtle populations from Bizerte (BM) and Haouaria (HM)

of new leaves. On the other hand, these two parameters were constant during the flowering and the fructification stages. Additionally, the length/width ratio of myrtle leaf was constant during the plant development having 2 for BM and 3 for HM. Similar results were obtained by (Mulas and Cani 1999). with leaf length/width ratio ranged from 1.6 to 3 for 70 wild Sardinian myrtle ecotypes.

3.1.1.3. Myrtle fruit

As for leaves, fruit length and width of the two populations were determined (Table 2). Although the fruit of BM had a length and width greater than HM throughout the maturation, there was a gradual increase in these dimensions reaching maximum lengths with 11.70 mm for HM and 11, 40 mm for BM as well as maximum widths of 8.30 mm for BM and 7.40 mm for HM in ripe fruits in January. Similar results were obtained by (Mulas and Cani 1999). who studied the fruit lengths (8-13 mm)

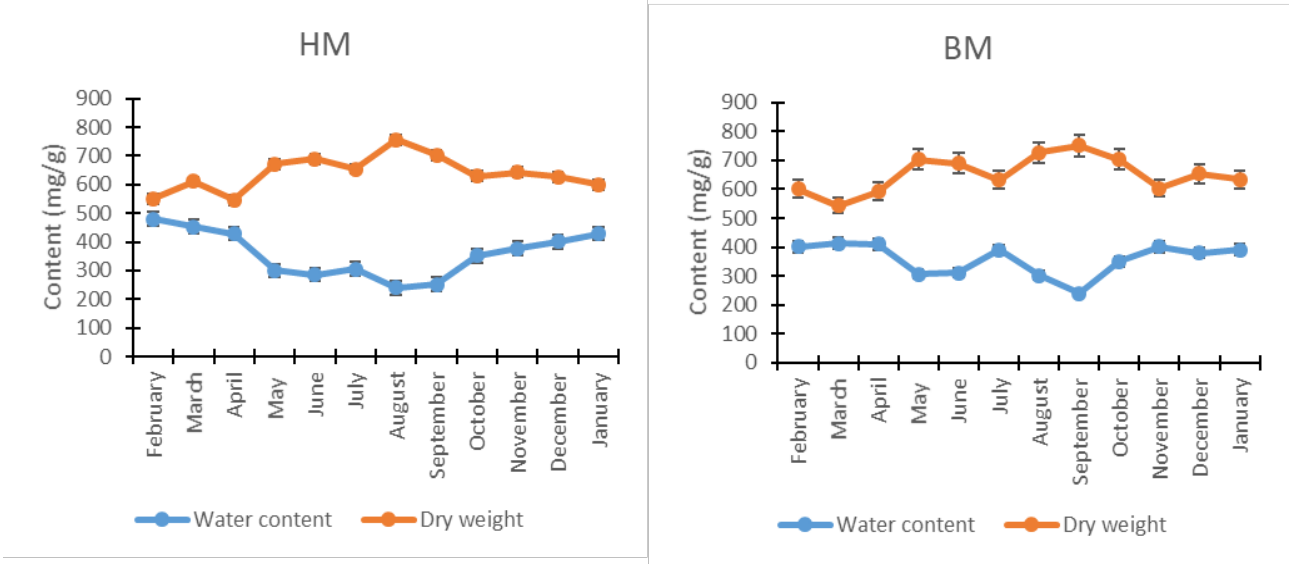

Fig. 3. Evolution of dry weight and water content of myrtle stem during development of two myrtle populations from Bizerte (BM) and Haouaria (HM)

and widths (6-13 mm) of 70 wild myrtle ecotypes from Sardinia. The ripe myrtle fruit of Turkey had a width (8.11 mm) comparable to that of BM fruit while longer (13.75 mm) than BM and HM fruits (Aydin and Öscan, 2006).

(Traveset et al. 2001) determined the characteristics of two types of myrtle fruits (dark blue (DB) and white (W) fruits). These fruit lengths were comparable to those studied at full maturity in our work (11.03 mm for DB and 10.87 mm for W) but with greater widths (10.21 mm for DB and 10.58 mm for W). According to (Fadda and Mulas 2010), Fruit development could be greatly influenced by climatic conditions. In fact, changes in

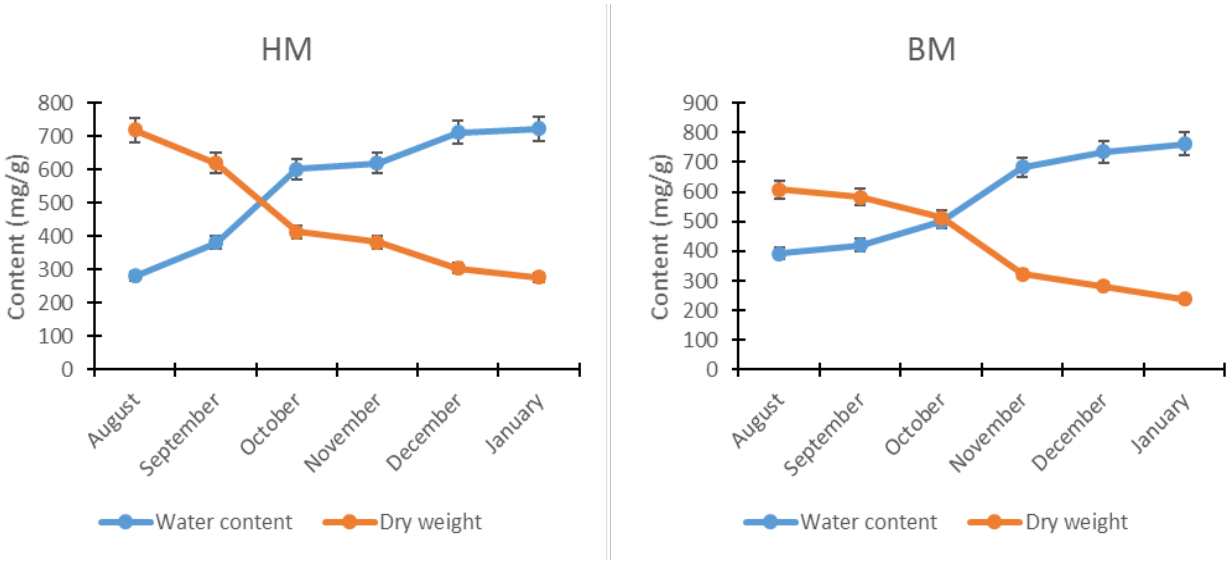

Fig. 4. Evolution of dry weight and water content of myrtle fruit during development of two myrtle populations from Bizerte (BM) and Haouaria (HM)

solar radiation, air temperature, rainfall distribution can affect the time at which myrtle fruit reach the final size.

3.1.2. Dry weight and water content

3.1.2.1. Myrtle leaf

The evolution of leaf water content during the vegetative cycle of each myrtle population was antagonist of that of leaf dry weight. A highly significant negative correlation was observed for both populations with r = -1 for HM and r = -0.955 for BM at p <0.05. It should be pointed out that the evolution of water content was inversely proportional to that of the dry weight throughout the vegetative cycle of the plants. The water content of *Myrtus communis* leaf showed a variable evolution for both populations (Fig. 2). An increase in water content during the fructification stage (from August to November) of 28.63% for HM and 36.77% for BM. This increase was also observed during the vegetative period (from February to May) of 2.14% for HM and 6.45% for BM whereas there was a decrease in the water content during the month of June and from July (flowering period) of 14.28% for HM and 13.38% for BM In a study of dry weight variation of *Thymbra spicata* and *Satureja thymbra*, (Müller-Riebau et al. 1997). also noted a decrease in leaf dry weight of leaves of both species during the growing season. According to (Bowler et al. 1992). this decrease in dry weight, accompanied by the increase in water content, may be due to the decrease in temperature.

3.1.2.2. Myrtle stem

The evolution of stem water content during the vegetative cycle of each myrtle population was opposed to that of dry weight (Fig. 3). The water content varied from 453.33 to 240.01 mg/g for HM and from 413.33 to 240.11 mg/g for BM while dry weight varied from 757.33 to 546.66 mg/g for HM and from 726.66 to 544.01 mg/g for BM.

3.1.2.3. Myrtle fruit

The evolution of dry weight and moisture content of the fruit of two populations of myrtle is shown in (Fig. 4) These two parameters had an opposite evolution during fruit ripening; there was a gradual increase in water content from 281 to 722.66 mg/g for HM and from 391.33 to 761.66 mg/g for BM in favor of a significant decrease in dry weight which varied from 719 to 277 mg/g for HM and 608.66 to 238.33 mg/g for BM. These results were comparable to those reported by (Aydin and Öscan 2006). who also studied the variation in water content of *Myrtus communis* fruit (83.2-744.4 mg/g). However, (Fadda and Mulas 2010). reported that dry weight of myrtle fruit rose from 19 to 130.7 mg for Barbara cultivar and from 12.5 to 237.7 mg for Daniela cultivar during maturation.

3.2. Biochemical characterization

3.2.1. Polyphenol determination

Given the importance of polyphenols as bioactive molecules, the evolution of these compounds at the level of the different organs of two myrtle populations from Bizerte and Haouaria was followed.

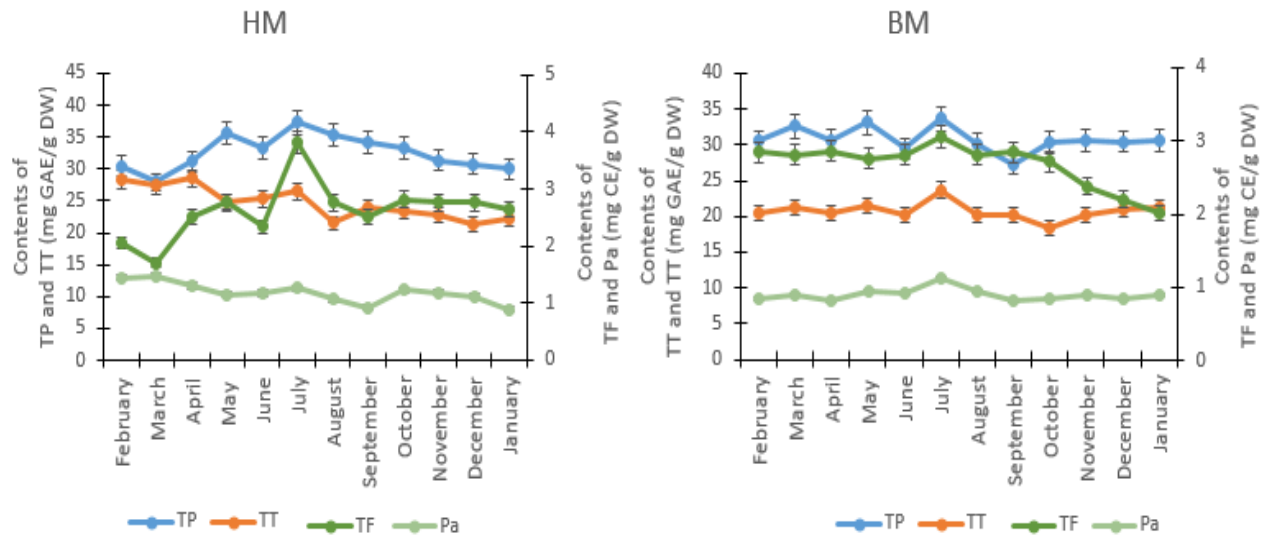

Fig. 5. Evolution of total polyphenol (TP), total tannin (TT), total flavonoid (TF) and proanthocyanidin (Pa)contents of myrtle leaf during the two population development (BM and HM).

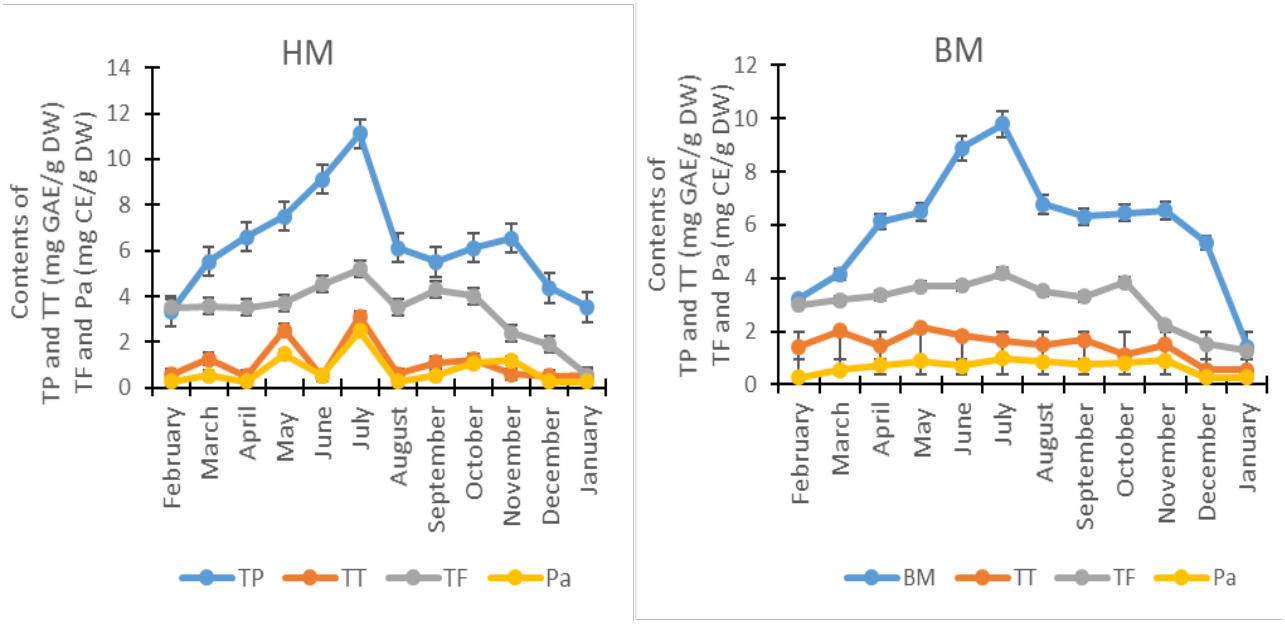

Fig. 6. Evolution of total polyphenol (TP), total tannin (TT), total flavonoid (TF) and proanthocyanidin (Pa) contents of myrtle stem during the two population development (BM and HM).

3.2.1.1. Myrtle leaf

The contents of total polyphenols, total tannins, total flavonoids and proanthocyanidins from myrtle leaf during the vegetative cycle of BM and HM populations are illustrated in (Fig. 5). The total polyphenol (TP) content of myrtle leaf in both populations significantly varied from 27.92 to 37.33 mg GAE/g DW for HM and 27.11 to 33.67 mg GAE/g DW for BM during the vegetative cycle. Maximum contents of TP were obtained at flowering during the month of July in both populations with 37.33 mg GAE/g DW for HM and 33.67 mg GAE/g DW for BM. However, these amounts slightly decreased during the other developmental stages having an average of 30 mg GAE/g DW in both populations. The total tannin (TT) contents of myrtle leaf in both populations showed the same trends as those of TP during the vegetative cycle of the two myrtle populations. Maximum contents of TT were obtained at bloom during the month of July in both populations with 26.55 mg GAE/g DW for HM and 23.67 mg GAE/g DW for BM. Beyond this period, there was a relative decrease in TT levels averaging 20 mg GAE / g DW in both populations. Regardless of stage of development and origin, the myrtle leaf was characterized by high levels of TT representing about 3/4 of the total polyphenols. The total flavonoid (TF) and proanthocyanidin (Pa) levels were also determined during the vegetative

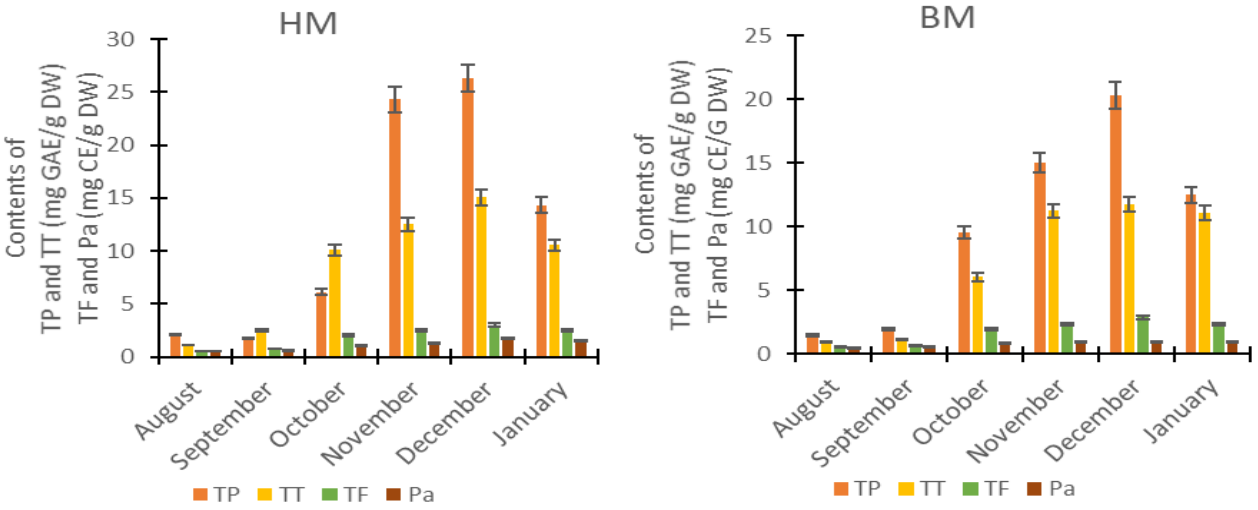

Fig. 7. Evolution of total polyphenol (TP), total tannin (TT), total flavonoid (TF) and proanthocyanidin (Pa)contents of myrtle fruit during the two population development (BM and HM).

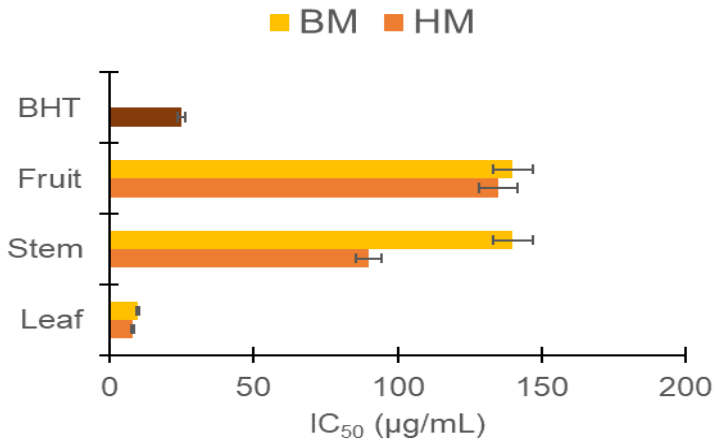

Fig. 8. Antiradical activity of methanol extract from different myrtle parts of Bizerte (BM) and Haouaria (HM) populations.

cycle of the two myrtle populations. TF contents were inversely related to those of TP, although they reached a maximum of 3 mg CE/g DW in both populations during the flowering period. Pa contents ranged from 0.97 to 1.22 mg CE/g DM for HM and 0.80 to 1.11 mg CE/g DW for BM. Regardless of development stage and origin, these two classes belong to the minor fraction of total polyphenols. Whatever the origin of myrtle, the amounts of the different phenolic categories as well as those of the total polyphenols were higher at the flowering period while undergoing a decrease during the vegetative stage and the fructification stage. This seasonal variation in total polyphenol contents was similar to that obtained by (Gardeli et al. 2008). for Greek myrtle but with contents 10 fold higher than that obtained in our study. Indeed, these authors noted that the total polyphenol contents reached a maximum at flowering (373 GAE / g DW) and a minimum at the vegetative stage (352 GAE/g DW) and at the fruiting stage (373 GAE/g DW). This significant increase during flowering coincided with the increase in the temperature of July. This supports the hypothesis of (Toor et al. 2006). suggesting that the accumulation of phenolic compounds taken place in order to preserve the plant from severe environmental conditions, especially abiotic factors such as salinity, ultraviolet radiation, high temperature and drought.

3.2.1.2. Myrtle stem

The TP, TF, TT and Pa contents of methanolic stem extracts during the vegetative cycle of two myrtle populations are marked in (Fig. 6). Stem TP contents significantly varied during the vegetative cycle of two myrtle populations ranging from 3.33 to 11.11 mg GAE/g DW for HM and 2.22 to 9.78 mg GAE/g DW for BM. Maximum contents of TP were obtained at flowering during the month of July with more than 9 mg GAE/g

DW for both populations. However, these amounts significantly decreased during the other developmental

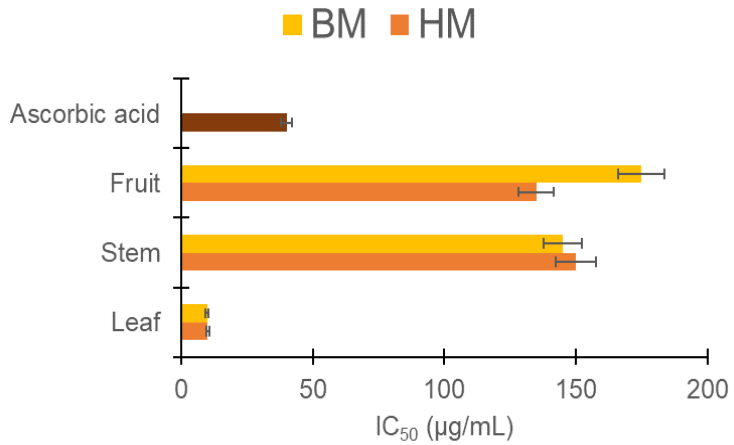

Fig. 9. Reducing power activity of methanol extract from different myrtle parts of Bizerte (BM) and Haouaria (HM) populations.

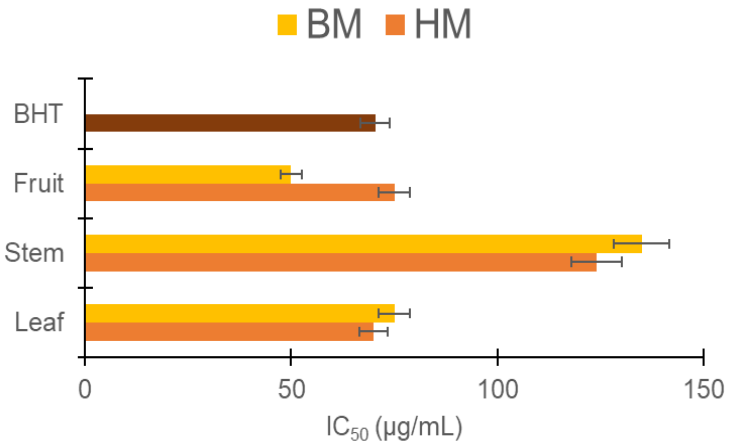

Fig. 10. β-carotene bleaching activity of methanol extract from different myrtle parts of Bizerte (BM) and Haouaria (HM) populations.

stages having an average of 3 mg GAE/g DW for both populations. The stem was richer in flavonoids than in condensed and hydrolysable tannins. TF amounts were also obtained at bloom during the month of July with 5.17 mg CE/g DW for both populations. Stem tannins belong to the minor fraction with slight variation during the vegetative cycle of two myrtle populations. It was determined that the variation of phenol, flavonoid and tannin content depended on the season which could be essential for acclimation of plants to seasonal changes in their environment (Medini et al. 2014).

3.2.1.3. Myrtle fruit

The TP, TF, TT and Pa contents of methanol fruit extracts from two myrtle populations during maturation are marked in (Fig. 7). During maturation, fruit TP amounts significantly varied of two myrtle populations with maximum levels at ripening stage during the month of December with more than 20 mg EAG/g DM for both populations. Maximum amounts of TF, Pa and TT were also obtained at ripening fruit stage during the month of December in both populations. In agreement to our results, an increase in TP contents was observed in sweet cherry (Serrano et al., 2005). Khirni (Patel and Rao, 2009). noni (Yang and Gadi, 2011). strawberry (Pinelli et al. 2011; Mahmood et al. 2012). and mulberry (Mahmood et al., 2012). fruits as maturity progressed. However, an inverse trend for TP contents was obtained for strawberry (Wang and Zheng, 2001). and acerola (Lima et al., 2005). fruits. It is interesting to mention that there was a decrease of TP content at the last stage of myrtle fruit maturity accordingly to (Fadda and Mulas 2010). Independently of maturation, myrtle fruit was richer in total tannins than TF and Pa, similarly to the results of (Aidi Wannes and Marzouk 2013).

3.2.2. Antioxidant activity

Taking into account the optimal period of biosynthesis of the polyphenols, the plant material used for the study of the antioxidant activity of the two myrtle populations

was collected during the flowering period for the leaf and stem and during the fruiting period for the fruit.

3.2.2.1. DPPH assay

Fig. 8 represents the antiradical activity of the different organs from two myrtle populations. Significant differences in the efficacy of the methanol extracts as a function of the organ were determined independently of myrtle origin. Methanol leaf extracts of the two myrtle populations had the higher antiradical potential than the synthetic antioxidant, BHT (IC_{50} = 25 µg/mL). However, the whole fruit and stem extracts of both populations had the weakest activities to inhibit DPPH with an IC_{50} exceeding 90 µg/mL. These results showed that the effect of myrtle origin was less marked than that of the organ. Indeed, the extracts obtained from both populations had similar antioxidant activities. (Gardeli et al. 2008). reported that the antiradical activity of methanol myrtle leaf extracts depended on the stage of development attending a maximum at the flowering period (IC_{50} = 9.54 µg/mL). (Hayder et al. 2004). who studied the effect of extraction solvent on the antiradical activity of Tunisian myrtle leaf extracts noted that the extracts with polar solvents exhibited the most important DPPH radical as the aqueous extract (IC_{50} = 1.3 µg/mL) and the methanol extract (IC_{50} = 6.5 µg/mL) whereas the extracts with apolar solvents (chloroform, hexane) have IC_{50} greater than 100 µg/mL.

3.2.2.2. Reducing power

In order to evaluate the reduction capacity of transition metal ions such as iron, measurement of the reducing power of methanol extracts of different organs of the two populations of myrtle was carried out (Fig. 9). Ascorbic acid was used as a positive control. The leaf was the most efficient organ to reduce Fe^{3+} ions with the lowest EC_{50} values ranging from 8 to 15 µg/mL in both populations. This organ had a greater reducing capacity than that of ascorbic acid (EC_{50} = 40 µg/mL), followed by that of the stem (EC_{50} varied from 132 to 154 µg/mL in both populations). However, methanol fruit extracts exhibited the lowest reducing power (EC_{50} = 130 µg/mL). In addition to the organ effect, results showed a significant effect of myrtle origin on this reducing activity. Indeed, the extracts of HM organs have the lowest EC50 values which vary from 8 to 150 µg / mL. It should be noted that there is a positive correlation between anti-radical and reductive activities which allowed us to deduce the hydrolysable tannins also have a significant reducing capacity of iron. (Gardeli et al. 2008). had demonstrated an important reducing power of the myrtle leaf that could be due to its richness in polyphenols. These metabolites represented hundreds of bioactive molecules whose main classes were phenolic acids, flavonoids and condensed and hydrolyzable tannins (Naczk and Shahidi, 2004).

3.2.2.3. β-carotene bleaching

As mentioned in Fig. 10, the inhibitory activity of β-carotene discoloration by the natural antioxidants of the various organ extracts was similar to that of the synthetic antioxidant (BHT) with the exception of the methanolic stem extracts which had the weakest inhibitory capacities with IC_{50} values greater than 150 µg/mL for both populations. The inhibitory power of β-carotene discoloration exerted by the methanolic extracts of the different organs of the two populations of myrtle. Indeed, (Narayan et al. 1999). noted that anthocyanins are able to inhibit both enzymatic and non-enzymatic lipid peroxidation reactions by the non-competitive, concentration-dependent mechanism. In addition, these authors have indicated that flavonoids can inhibit the lipid peroxidation process by trapping free radicals.

4. Concluding remarks

In conclusion, it should be pointed out that, although these two populations showed similar phenological dynamism, some morphological differences have been recorded, such as the great shape of BM leaf and fruit. The production of phenolic compounds was high during flowering in both populations as well as there was a significant variation in their levels in different organs. During this period, the antioxidant activity of the various organs, particularly the leaf, was important.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Agrimonti, C., Bianchi, R., Bianchi, A., Ballero, M., Poli, F., Marmiroli, N., 2007. Understanding biological conservation strategies: A molecular-genetic approach to the case of myrtle (*Myrtus communis* L.) in two Italian Regions: Sardinia and Calabria. Conserv. Genet. 8(2), 385-396.

Aidi Wannes, A., Mhamdi, B., Sriti, J., Ben Jemia, M., Ouchikh, O., Hamdaoui, G., Kchouk, M.E., Marzouk, B., 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. Food Chem. Toxicol. 48(5), 1362-1370.

Aidi Wannes, W., Marzouk, B., 2013. Differences between myrtle fruit parts (*Myrtus communis* var. *italica*) in phenolics and antioxidant contents. J. Food Biochem. 37(5), 585-594.

Aidi Wannes, W., Mhamdi, B., Marzouk, B., 2007. Essential oil composition of two *Myrtus communis* L. varieties grown in North Tunisia. Ital. J. Biochem. 56(2), 180-186.

Aidi Wannes, W., Mhamdi, B., Marzouk, B., 2009. Variations in essential oil and fatty acid composition during *Myrtus communis* var. *italica* fruit maturation. Food Chem. 112(3), 621-626.

Aidi Wannes, W., Marzouk, B., 2012. Maturational effect on essential oil yield and composition of *Myrtus*

communis var. *baetica* fruit. J. Essent. Oil bear. Pl. 15(5), 847-853.

Aidi Wannes, W., Marzouk, B., 2016. Characterization of myrtle seed (*Myrtus communis* var. baetica) as a source of lipids, phenolics, and antioxidant activities. J. Food Drug Anal. 24(2), 1-8.

Aidi Wannes, W., Mhamdi, B., Sriti, J., Bettaieb, I., Saidani Tounsi, M., Marzouk, B., 2011. Fatty acid and glycerolipid changes during Tunisian myrtle (*Myrtus communis* var. *italica*) fruit ripening. J. Food Biochem. 35(1), 177-194.

Aleksic, V., Knezevic, P., 2014. Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. Microbiol. Res. 169(4), 240-254.

Anwar, S, Crouch, R.A., Awadh Ali, N.A., Al-Fatimi, M.A., Setzer, W.N., Wessjohann, L., 2017. Hierarchical cluster analysis and chemical characterisation of *Myrtus communis* L. essential oil from Yemen region and its antimicrobial, antioxidant and anti-colorectal adenocarcinoma properties. Nat. Prod. Res. 31(18), 2158-2163.

Aydin, C., Özcan, M.M., 2007. Determination of nutritional and physical properties of myrtle (*Myrtus communis* L.) fruits growing wild in Turkey. J. Food. Eng. 79(2), 453-458.

Barboni, T., Venturini, N., Paolini, J., Desjobert, J.M., Chiaramonti, N., Costa, J., 2010. Characterisation of volatiles and polyphenols for quality assessment of alcoholic beverages prepared from Corsican *Myrtus communis* berries. Food Chem. 122(4), 1304-1312.

Bowler, C., Van Montagu, M., Inze, D., 1992. Superoxyde dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43, 83-116.

Cannas, S., Molicotti, P., Ruggeri, M., Cubeddu, M., Sanguinetti, M., Marongiu, B., Zanetti, S., 2013. Antimycotic activity of *Myrtus communis* L. towards Candida spp. from clinical isolates. J. Infect. Dev. Ctries, 7(3), 295-298.

Dewanto, V., Wu, X., Adom, K.K., Liu, R.H., 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50(10), 3010-3014.

Fadda, A., Mulas, M., 2010. Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening. Sci. Horticult. 5(3), 477-485.

Gardeli, C., Papageorgiou, V., Mallouchos, A., Theodosis, K., Komaitis, M. 2008. Essential oil composition of Pistacia lentiscus L. and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts. Food Chem. 107(3), 1120-1130.

Hagerman, A.E., Butler, L.G., 1978. Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem. 26(4), 809-812.

Hanato, T., Kagawa, H., Yasuhara, T., Okuda, T., 1988. Two new flavonoids and other constituents in licorice root their relative astringency and radical scavenging effect. Chem. Pharmt. Bull. 36(6), 1090-1097.

Hayder, N., Abdelwahed, A., Kilani, S., Ben Ammar, R., Mahmoud, A., Ghedira, K., Chekir Ghedira, L., 2004. Antimutagenic and free radical scavenging activities of extracts from (Tunisian) *Myrtus communis*. Mutat. Res. 564(1), 89-95.

Khan, N., Rasool, S., Ali Khan, S., Bahadar Khan, S., 2019. A new antibacterial dibenzofuran-type phloroglucinol from *Myrtus communis* linn, Nat. Prod. Res., doi: 10.1080/14786419.2018.1556657.

Koleva, I.I., Teris, A.B., Jozef, P.H., Linssen, A.G., Lyuba, N.E., 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13(1), 8-17.

Lima, V.L.A.G., Melo, E.A., Maciel, M.I.S., Prazeres, F.G., Musser, R.S., Lima, D.E.S., 2005. Total phenolic and carotenoid contents in acerola genotypes harvested at three ripening stages. Food Chem. 90(4), 565-568.

Mahmood, T., Anwar, F., Abbas, M., Saari, N., 2012. Effect of maturity on phenolics (phenolic acids and flavonoids) profile of strawberry cultivars and mulberry species from Pakistan. Int. J. Mol. Sci. 13(4), 4591-4607.

Marchiori, N.C., Sobral, M., 1997. 'Dendrologia das angiospermas: Myrtales. Santa Maria: Ed. da JFSM, 304 p.

Mau, J.L., Chao, G.R., Wu, K.T., 2001. Antioxidant properties of methanolic extracts from several ear mushrooms. J. Agric. Food Chem. 49(11), 5461-5467.

Medini F., Fellah H., Ksouri R., Abdelly, C., 2014. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. J. Taibah Univ. Sci. 8(3), 216-224.

Melito S., La Bella S., Martinelli F., Cammalleri I., Tuttolomondo T., Leto C., Fadda A., Molinu M.G., Mulas M., 2016. Morphological, chemical, and genetic diversity of wild myrtle (*Myrtus communis* L.) populations in Sicily. Turk. J. Agric. For. 40, 249-261.

Messaoud C., Khoudja M., Boussaid M., 2006. Genetic diversity and structure of wild Tunisian *Myrtus communis* L. (Myrtaceae) populations. Genet. Resour. Crop. Evol. 53(2), 407-417

Messaoud, C., Zaouali, Y., Ben Salah, A., Khoudja, M.L., Boussaid, M. (2005) *Myrtus communis* in Tunisia: Variability of the essential oil composition in natural populations. Flav. Fragr. J. 20(6), 577-582.

Mulas, M., 2012. The myrtle (*Myrtus communis* L.) case, from a wild shrub to a new fruit crop. Acta Horticult. 948(27), 235-242.

Mulas, M., Cani, M.R., 1999. Germoplasm evaluation of spontaneous myrtle (*Myrtus communis* L.) for cultivar selection and crop development. J. Herbs Spices Med. Plants 6(3), 31-49.

Müller-Riebau, F.J., Berger, M.B., Yegen, O., Cakir, C., 1997. Seasonal variations in the chemical compositions of essential oils of selected aromatic plants growing wild in Turkey. J. Agric. Food Chem. 45(12), 4821-4825.

Naczk, M., Shahidi, F., 2004. Extraction and analysis of phenolics in food. J. Chromatogr. 1054(1-2), 95-111.

Narayan, M.S., Naidu, K.A., Ravishankar, G.A., Srinivas, L., Venkataraman, L.V., 1999. Antioxidant effect of anthocyanin on enzymatic and non-enzymatic lipid peroxidation. Prostaglandins Leukot. Essent. Fatty Acids 60(1), 1-4.

Oyaizu, M., 1986. Studies on products of the browning reaction: Antioxidative activities of browning reaction. Jap. J. Nutr. 44(6), 307-315.

Panjeshahin, M.R., Azadbakht, M., Akbari, N., 2016. Atidiabetic activity of different extracts of *Myrtus communis* in streptozotocin induced diabetic rats. Rom. J. Diabetes Nutr. Metab. Dis. 23(2), 183-190.

Patel, P.R., Rao, T.V.R., 2009. Physiological changes in relation to growth and ripening of khirni [Manilkara hexandra (Roxb.) Dubard] fruit. Fruits 64(3), 139-146.

Pineli, L.L.O., Moretti, C.L., Santos, M.S., Campos, A.B., Brasileiro, A.V., Cordova, A.C, Chiarello, M.D., 2011. Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. J. Food Compos. Anal. 92(1), 831-838.

Pottier Alapetite, G., 1979. Flore de la Tunisie, Première partie. Eds., *Publications Scientifiques Tunisiennes*, Tunis, 654 p.

Sanna, D, Mulas, M, Molinu, M.G., Fadda, A., 2019. Oxidative stability of plant hydroalcoholic extracts assessed by EPR spin trapping under forced ageing conditions: A myrtle case study. Food Chem. 271, 753-761.

Scazzocchio, F., Garzoli, S., Conti, C., Leone, C., Renaioli, C., Pepi, F., Angiolella, L., 2016. Properties and limits of some essential oils: chemical characterisation, antimicrobial activity, interaction with antibiotics and cytotoxicity. Nat. Prod. Res. 30(17), 1909-1918.

Serrano, M., Guillen, F., Martinez-Romero, D., Castillo, S., Valero, D., 2005. Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. J. Agric. Food Chem. 53(7), 2741-2745.

Serce, S., Ercisli, S., Sengul M., Gunduz, K., Orhan, E., 2010. Antioxidant activities and fatty acid composition of wild grown myrtle (*Myrtus communis* L.) fruits. Pharmacognosy Med. 6(21), 9-12.

Sun, B., Richardo-da-Silvia, J.M., Spranger, I., 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 46(10), 4267-4274.

Talebianpoor, M.S., Talebianpoor, M.S., Mansourian, M., Vafaiee-Nejad, T., 2019. Antidiabetic activity of hydroalcoholic extract of *Myrtus communis* (myrtle) fruits in streptozotocin-induced and dexamethasone-induced diabetic rats. Pharmacognosy Res. 11(2), 115-120.

Tardugno, R., Pellati, F., Iseppi, R., Bondi, M., Bruzzesi, G., Benvenuti, S., 2018. Phytochemical composition and *in vitro* screening of the antimicrobial activity of essential oils on oral pathogenic bacteria. Nat. Prod. Res. 32(5), 2018.

Toor, R.K., Savage, G.P., Lister, C.E., 2006. Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. J. Food Compos. Anal. 19(1), 1-10.

Traveset, A., Riera, N., Mas, R.E., 2001. Ecology of fruit-colour polymorphism in *Myrtus communis* and differential effects of birds and mammals on seed germination and seedling growth. J. Ecol. 89(5), 749-760.

Tuberoso, C.I.G., Barra, A., Angioni, A., Sarritzu, E., Pirisi, F.M., 2006. Chemical composition of volatiles in Sardinian myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils. J. Agric. Food Chem. 54(4), 1420-1430.

Tuberoso, C.I.G., Melis, M., Angioni, A., Pala, M., Cabras, P., 2007. Myrtle hydroalcoholic extracts obtained from different selections of *Myrtus communis* L. Food Chem, 101(2), 806-811.

Usai, M., Marchetti, M., Mulas, M., 2015. Chemical composition of essential oils of leaves and flowers from five cultivars of myrtle (*Myrtus communis* L.). J. Essent. Oil Res. 27(6), 465-476.

Usai, M., Marchetti, M., Culeddu, N., Mulas M., 2018. Chemical composition of myrtle (*Myrtus communis* L.) berries essential oils as observed in a collection of genotypes. Molecules 23, 1-20.

Wang, S.Y., Zheng, W., Effect of plant growth temperature on antioxidant capacity in strawberry. J. Agric. Food Chem. 49(10), 4977-4982.

Yang, J., Gadi, R., Thomson, T., 2011. Antioxidant capacity, total phenols, and ascorbic acid content of noni (*Morinda citrifolia*) fruits and leaves at various stages of maturity. Micronesica 41(2), 167-176.