CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS AND ETHANOL EXTRACTS OF *MYRTUS COMMUNIS* L. ORGANS (BERRIES, LEAVES AND FLORAL BUDS)

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ABSTRACT: In this study, we compared the chemical composition and antioxidant activities of essential oils and ethanol extracts from *Myrtus communis* L. berries, leaves, and floral buds. The chemical composition of essential oils was analysed using gaz chromatography (GC) and gaz chromatography coupled to mass spectrometry (GC-MS). The oils comprised mainly monoterpenes hydrocarbons and their oxygenated derivatives with a large amount of α-pinene, the main compound of the berries and floral buds oils (34.3 and 48.9% respectively). In leaf oil, 1,8-cineole was more abundant (61.0 %). The amounts of total polyphenolics, flavonoids and proanthocyanidins in ethanol extracts were determined by spectrophotometry. Differences were found in their amounts among the studied organs. The antioxidant activities of the essential oils and ethanol extracts were evaluated by the di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) radical scavenging test and the β-carotene bleaching method. Results showed that ethanol extracts of all different organs exhibited higher antioxidant activity than essential oils. Floral buds ethanol extracts showed the highest activity.

Keywords: *Myrtus communis* L., organs, essential oil, ethanol extract, antioxidant activity.

1. INTRODUCTION

Essential oils and extracts obtained from many plants have recently received great attention and scientific interest due to their potential effects on the reactive oxygen species (ROS) involved in several degenerative diseases [1], including cancers [2], cardiovascular, neurodegenerative [3] and inflammatory diseases [4]. Moreover, synthetic antioxidants, such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) are suspected of being responsible for severe toxic effects and health risks [5]

Aromatic and medicinal plants contain a wide variety of natural antioxidants such as polyphenolic acids, flavonoids, tannins and essential oils. They possess more potent antioxidant activity than dietary plants [6,7]. In Tunisia, more than 25% of the spontaneous flora is recognised as having medicinal and aromatic properties [8]. Among this species, *Myrtus communis* L. commonly named myrtle is considered as the most representative one. It is a pleasant shrub or small tree with dense foliage belonging to the Myrtaceae family. It grows mainly under Quercus suber L. and Q. faginea Lamk. forests in humid and sub-humid bioclimatic stages [8]. Different parts of *Myrtus communis* L. such as leaves, fruits, flowers and roots has been used for medicinal, food, spices and cosmetic purposes [9]. Several studies have confirmed the antioxidant [10,11,12,13,14], antimicrobial and

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antifungal [15] properties of myrtle extracts and essential oils. Recently, anticancer activity of some myrtle compounds was assessed [16].
Variation in chemical composition of essential oils and extracts of medicinal plants may be observed due to the origin, the environmental conditions and the nature of plant organ. Therefore, antioxidant and other biological activities mainly attributed to the active compounds of their essential oils and polyphenolic fractions may vary because of the variations in the chemical composition [17].
The aim of this work was to compare the chemical composition and the antioxidant properties of essential oils (EOs) and ethanol extracts (EEs) from three myrtle organs: berries (B), leaves (L) and floral buds (FB) as well as to acquire valuable data about the best organ and procedure to use in order to obtain extracts containing active principles.

2. MATERIAL AND METHODS

2.1. Reagents

Folin-Ciocalteu reagent, β-carotene, *cis, cis*-9,12-Octadecadienoic acid (linoleic acid), di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH), 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT), aluminium chloride ($AlCl_3$), hydrochloric acid (HCl), 3,4,5-trihydroxybenzoic acid (gallic acid), quercetin, catechin were procured from Sigma-Aldrich Chemie. Analytical grade ethanol, chloroform and Tween 40 were obtained from Merck. α-Thujene, α-pinene, camphene, β-pinene, myrcene, α-terpinene, limonene, γ-terpinene, terpinolene, 1,8-cineole, linalool, borneol, linalyl acetate, eugenol, methyleugenol, alkane standard solutions (C_8-C_{24}) were from Fluka Chemika.

2.2. Plant materials

Myrtus communis L. samples were collected from plants grown in the region of Ain Draham (North West of Tunisia). Berries (B) were collected in January 2010, while floral buds (FB) and leaves (L) were harvested in June 2010. The harvested plants were identified according to Pottier-Alapetite [18]. Voucher specimens were deposited in the herbarium of the High School of Food Industries for future reference. Leaves and floral buds were dried at room temperature under dark conditions and berries were packaged under vaccum and stored at -18°C.

2.3. Isolation of the essential oils (EOs)

Samples of 150 g of each organ were submitted to hydrodistillation using Dean-Stark apparatus,for 90 min until there was no significant increase in the volume of oil collection. Afterward, the essential oil was dried over sodium sulfate anhydrous for 15 min and stored in a sealed vial at 4 °C prior to analysis.

2.4. GC and GC-MS analysis

GC analyses were performed using a Hewlett-Packard 6890 series gas chromatograph, equipped with a flame ionization detector. A 30 m HP-5MS (5% phenylmethylsiloxane) capillary column, 0.25mm i.d. and 0.25 µm film thickness was employed. Helium was used as a carrier gas at a flow rate of 0.9 $mL.min^{-1}$. the temperatures of injector and detector were set at 250 °C and 280 °C, respectively. Oven temperature program was: 40 °C for 1 min, increased to 250 °C at 2 °C min^{-1}, held for 5 min. Samples were injected into GC using the split mode with a split ratio of 1/10. The GC-MS analysis was carried out on a HP 6890 instrument coupled to a HP 5973N MS computerized system, and equipped with HP-5MS column with the same characteristics as the one used in GC. The ion source temperature was 230°C. The ionization energy was 70eV with a scan of 1 s and mass range of 40-300 amu. The percentage of the compounds was calculated from the GC peak areas, using the normalization method.
Compounds were identified by comparison of their mass spectra with those in the Wiley 238.L mass spectra library. The obtained compounds were also confirmed by comparing their retention indices determined by co-injection of the sample with a solution containing the homologous series of C8-C22 *n*-alkanes with the data published in the literature [19,20], and whenever possible by co-injection with a internal standard.

2.5. Preparation of ethanol extracts (EEs)

Fifty grams of each organ were extracted separately for three times with 80 % aqueous ethanol (3 x 300 mL) by agitated maceration at room temperature every 24h the solvent is removed. The extracts obtained from

three extractions of each organ were combined, filtered through a Whatman No.4 filter paper (porosity of 25µm) and concentrated under reduced pressure. EEs were stored at 4 °C until analysis.

2.6. Total polyphenolic content

Total polyphenol content was determined with the Folin–Ciocalteu reagent using the method of Lister and Wilson [21]. 100 µL of the diluted sample were dissolved in 500 µL (1/10 dilution) of the Folin–Ciocalteu reagent and 1 mL of distilled water.

The solutions were mixed and incubated at room temperature. After 1 min, 1.5 mL of 20 % sodium carbonate solution were added. The final mixture was shaken thoroughly and then incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm and results were expressed in mg of gallic acid equivalents per gram (mg GAE.g^{-1}).

2.7. Total flavonoid content

The $AlCl_3$ method [22] was adapted for the purpose of determining the total flavonoid content of the ethanol extracts. 1.5 mL of extracts was added to equal volumes of a solution of 2% $AlCl_3.6H_2O$. The mixture was thoroughly mixed and incubated for 10 min at room temperature; the absorbance was read at 367.5 nm. Data were expressed in mg quercetin equivalents per gram (mg QE.g^{-1}).

2.8. Total proanthocyanidins content

The HCl/butan-1-ol assay was used to quantify total proanthocyanidins [23]. 0.25 mL of extract was added to 3 mL of a 95% solution of n-Butanol/HCl (95:5 v/v) and 0.1 mL of a solution of $NH_4Fe(SO_4)_2.12H_2O$ in 2 M HCl in stoppered test tubes. The tubes were incubated for 40 min at 95 °C. The absorbance of the red color was read at 550 nm with data expressed as mg catechin equivalents per gram (mg CE.g^{-1}).

2.9. DPPH assay

The anti-radical activities of EOs and EEs were evaluated using the test of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [12]. 2 mL of different concentrations of EOs and EEs in ethanol and 2 mL of ethanol for control sample were mixed with 2 mL of freshly prepared DPPH solution in ethanol (2.10^{-4} M) and allowed to stand for 30 min in the dark at room temperature. The absorbance of the solution was measured at 517 nm against ethanol as the blank. The radical scavenging activity was expressed as IC_{50} (µg.mL^{-1}), the concentration providing 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following formula: %inhibition = $[(AC_{(0)} - AS_{(t)})/AC_{(0)}] \times 100$, where $AC_{(0)}$ is the absorbance of the control at t = 30 min and $AS_{(t)}$ is the absorbance of the tested sample at t = 30 min. BHT was used as a positive control. Tests were carried out in triplicate.

2.10. β-carotene bleaching method

The antioxidant activities of EOs and EEs were estimated using β-carotene bleaching method [12]. Briefly, 2 mg of β-carotene was dissolved in 10 mL chloroform. The carotene - chloroform solution, 1 mL, was mixed with 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator at 40 °C for 5 min. 50 mL of oxygenated distilled water was added to the residue slowly with vigorous agitation, to form an emulsion. The emulsion (4 mL) was added to tubes containing 0.2 mL of the sample solution in ethanol (2 mg.mL^{-1}), 0.2 mL of ethanol for negative control and 0.2 mL of BHT for positive control. The blank consisted on an emulsion without β-carotene. The absorbance was immediately measured at 470 nm. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm until the color of β-carotene disappeared in the control (t = 120 min). Antioxidant activity percentages (%AA) were calculated using the following equation: $\%AA = [(AS_{(120)} - AC_{(120)}) / (AC_{(0)} - AC_{(120)})] \times 100$, where $AS_{(120)}$ is the absorbance of the tested sample at 120 min, $AC_{(120)}$ is the absorbance of the control at 120 min and $AC_{(0)}$ is the absorbance of the control at 0 min. The tests were carried out in triplicate.

2.11. Statistical analysis

Results were expressed as means ± SD. Differences were tested for significance by the analysis of variance procedure (Statgraphics Centurion XVI) using a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of EOs

EOs yields varied significantly between myrtle organs with 0.02% for berries, 0.5% for leaves and 0.2% for floral buds (Figure 1). So the highest EO yield was observed for leaves while the lowest for berries.

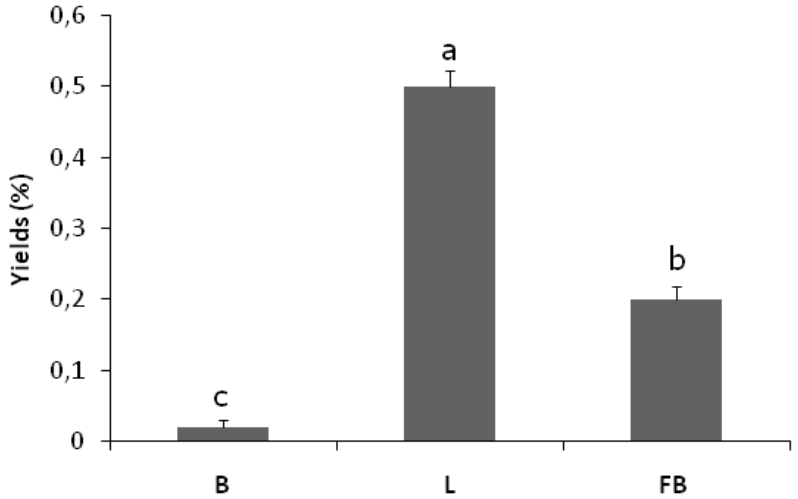

Figure 1. Essential oil yields of berries (B), leaves (L) and floral buds (FB) of *Myrtus communis* L. Data are given as mean ± SD (n=3)

The EOs were analysed by GC and GC/MS. Thirty two, twenty three and thirty seven constituents were identified and quantified respectively in berries, leaves and floral buds (Table I). Results showed that the chemical composition of the studied oils were rather close, being dominated by monoterpenes hydrocarbons (25.7–68.3%) and oxygen-containing monoterpenes (27.0–70.0%). We note, however that the composition presented differences between leaves oils to floral buds ones. In fact, α-pinene is the major compound found in berries and floral buds (34.3 and 48.9 % respectively). While 1,8-cineole was the major component of leaves EO with 61.0 % of the total composition. Aliphatic, benzenoid and sesquiterpenoid compounds were more abundant in berries oil representing 4.2, 2.5 and 2.2 % respectively.

Our results were coherent with those of Wannes et al. [14] who reported a variation in yields and chemical composition of EOs from myrtle leaf, stem and flower. The noticeable differences in the chemical composition of the oils isolated from berries, leaves and floral buds of *Myrtus communis* L. may be explained by diverging biosynthetic pathways of volatile compounds in the respective plant part [24]. It is also important to mention that differences in the yield and composition can be partly explained by the differences in secretory organs structures [25]. The results confirm other authors works that found α-pinene and 1,8-cineole being the major constituents of Tunisian myrtle oils [14,26,27].

Table I. Essential oil composition (%) of berries (B), leaves (L) and floral buds (FB) of *Myrtus communis* L.

Compound[a]	RI[b]	Identification[c]	Percentage[d]		
			B	L	FB
Ethyl isobutyrate	761	RI, MS	-	-	0.2
Isobutyl isobutyrate	921	RI, MS	1.4	0.6	-
Tricyclene	924	RI, MS	-	-	0.1
α-Thujene	928	RI, MS, CoI	-	0.2	0.5
α-Pinene	938	RI, MS, CoI	34.3	23.7	48.9

α-Fenchene	948	RI, MS	-	-	0.2
Camphene	950	RI, MS, CoI	0.4	-	0.1
Sabinene	972	RI, MS	-	-	0.4
β-Pinene	980	RI, MS, CoI	0.5	0.5	0.1
Myrcene	991	RI, MS, CoI	0.2	0.2	0.1
Isobutyl 2-methylbutyrate	1010	RI, MS	2.2	0.4	0.5
δ-3-Carene	1012	RI, MS	-	0.5	1.6
2-Methylbutyl isobutyrate	1014	RI, MS	0.6	-	-
α-Terpinene	1018	RI, MS, CoI	-	-	0.4
p-Cimene	1025	RI, MS	-	-	2.0
Limonene	1032	RI, MS, CoI	-	-	6.5
1,8-Cineole	1033	RI, MS, CoI	26.6	61.0	15.3
(Z)-β-Ocimene	1040	RI, MS	-	-	0.1
(E)-β-Ocimene	1048	RI, MS	-	-	2.1
γ-Terpinene	1063	RI, MS, CoI	0.6	0.4	2.5
Terpinolene	1093	RI, MS, CoI	0.8	0.2	2.7
Linalool	1101	RI, MS, CoI	5.9	1.7	3.1
trans-Pinocarveol	1139	RI, MS	0.2	0.3	-
p-Mentha-1,5-dien-8-ol	1142	RI, MS	-	0.2	-
Borneol	1163	RI, MS, CoI	0.2	-	0.2
Terpinen-4-ol	1179	RI, MS	0.5	0.8	0.3
α-Terpineol	1189	RI, MS	4.4	3.3	0.8
Myrtenol	1202	RI, MS	-	-	2.7
Methyl chavicol	1205	RI, MS	0.3	-	-
Nerol	1228	RI, MS	1.2	-	-
Carvone	1241	RI, MS	1.9	-	-
Citral	1237	RI, MS	0.3	-	-
Geraniol	1257	RI, MS	0.3	0.6	2.3
Linalyl acetate	1262	RI, MS, CoI	-	-	1.8
exo-2-Hydroxycineole acetate	1354	RI, MS	0.2	0.2	-
Eugenol	1357	RI, MS, CoI	-	-	1.1
Neryl acetate	1368	RI, MS	0.4	-	0.2
Geranyl acetate	1384	RI, MS	4.5	1.9	0.3
β-Elemene	1392	RI, MS	-	-	0.1
Methyl eugenol	1404	RI, MS, CoI	2.2	0.3	0.6
β-Caryophyllene	1419	RI, MS	0.8	0.3	0.2
γ-Elemene	1433	RI, MS	-	-	0.1
α-Humulene	1454	RI, MS	0.5	0.1	-
allo-Aromadendrene	1460	RI, MS	-	-	0.1
α-Curcumene	1481	RI, MS	0.4	-	-
α-Zingiberene	1495	RI, MS	0.7	-	-
β-Bisabolene	1510	RI, MS	0.3	-	-
Geranyl isobutyrate	1516	RI, MS	-	0.1	-
Spathulenol	1576	RI, MS	-	-	0.3
Caryophyllene oxyde	1584	RI, MS	0.6	0.3	0.1
humulene Epoxyde II	1603	RI, MS	-	-	0.1
β-Eudesmol	1648	RI, MS	0.2	-	-
α-Cadinol	1653	RI, MS	0.3	-	-
Classes					
Aliphatic compounds			4.2	1.1	0.7
Monoterpene hydrocarbons			36.8	25.7	68.3

Oxygen-containing monoterpenes	46.6	70.0	27.0
Benzenoid compounds	2.5	0.3	1.7
Sesquiterpe hydrocarbons	3.8	0.7	1.0
Total (%)	**93.9**	**97.8**	**98..7**

[a] Compounds listed in order of elution from HP-5MS column.
[b] Retention indices relative to C8 – C22 *n*-alkanes on HP-5MS column.
[c] RI: Retention indices relative to C8 – C22 *n*-alkanes on HP-5MS column, MS: mass spectrum, CoI: co-injection with authentic compounds (Fluka chemika).
[d] Percentage (mean of three analyses) based on FID peak area

3.2. Contents of total polyphenols, flavonoids and proanthocyanidins

The amounts of total polyphenols, flavonoids and proanthocyanidins of the different ethanol extracts are shown in figure 2.

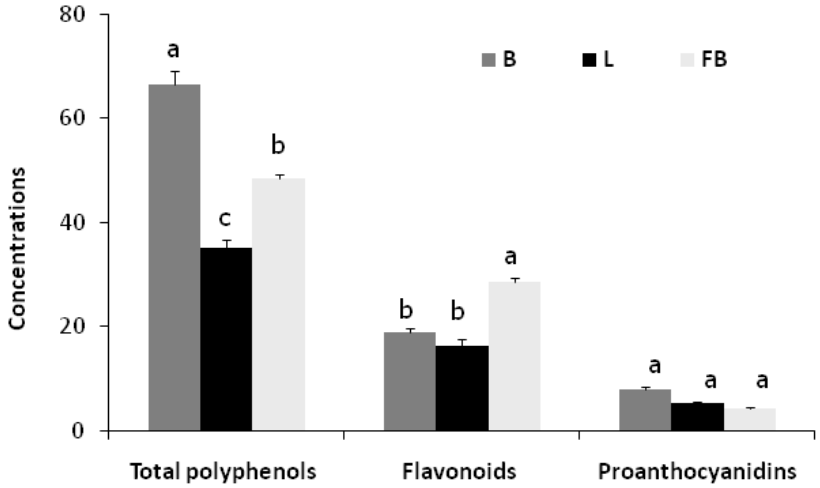

Figure 2. Total polyphenols (mg GAE/g), flavonoids (mg QE/g) and proanthocyanidins (mg CE/g) contents in berries (B), leaves (L) and floral buds (FB) of *Myrtus communis* L. Data are given as mean ± SD (n=3)

All myrtle organs showed high total polyphenols contents. Berries recorded the highest levels (66.5 mg GAE.g^{-1}), 1.5 times higher than floral berries (48.3 mg GAE.g^{-1}) and two times higher than leaves (35.2 mg GAE.g^{-1}).

Flavonoid contents in the studied organs varied from 16.2 to 28.6 mg QE.g^{-1}. It was higher in floral buds (28.6 mg QE.g^{-1}) than berries (18.8 mg QE.g^{-1}) and leaves (16.2 mg QE.g^{-1}). Proanthocyanidins were also present in the studied extracts. Although, in lower abundance than flavonoids. Berries (B) showed higher proanthocyanidin contents (7.9 mg CE.g^{-1}) than did leaves and floral buds (5.2 and 4.3 mg CE.g^{-1} respectively).

These results are in agreement with Amensour et al. [10] and Wannes et al. [14] who confirmed that the distribution of myrtle polyphenolic compounds were organ dependent. Nevertheless, differences in the obtained amounts were observed. This may be related mainly to the environmental conditions. Indeed, the biosynthesis and the accumulation of polyphenolic compounds in a plant depend on a number of intrinsic (genetic) and extrinsic (environmental, handling and storage) factors [28].

3.3. Antioxidant activity

The antiradical activities of each of the considered EOs and EEs were measured as their IC_{50} through *in vitro* test of the stable radical DPPH. The IC_{50} value is negatively related to the antioxidant activity, the lower the IC_{50} value, the higher the antioxidant activity of the tested

sample. Results showed that DPPH radical scavenging ability depends on both the used part of the plant and the extraction method (Table II). EEs of all myrtle parts exhibited stronger activity than EOs. EE_FB is the most potent radical scavenger with IC_{50} = 21 µg.mL^{-1}, comparable to that of synthetic antioxidant BHT (IC_{50} = 20 µg.mL^{-1}), followed by EE_B (IC_{50} = 42 µg.mL^{-1}) and EE_L (IC_{50} = 70.5 µg.mL^{-1}). The same order was observed with EOs of different myrtle parts with IC_{50} values of 240, 524 and 941 µg.mL^{-1} for floral buds, berries and leaves respectively.

Table II. Antioxidant activities of essential oils s and ethanol extracts of berries (B), leaves (L) and floral buds (FB) of *Myrtus communis* L. Values are given as mean ± SD (n=3).

	DPPH assay IC_{50} (µg.mL^{-1})	**β-carotene method %AA**
Essential oil		
Berries	524.0±2	34.5± 2
Leaves	941.0±2	19.4±1
Floral buds	240.0±3	67.0±2
Ethanol extract		
Berries	42.0±2	54.5±2
Leaves	73.0±2	41.7±1
Floral buds	21.0±0.2	70.5±3
BHT	20.0±1	58.2±1

The antioxidant activity of EOs and EEs was also evaluated by the β-carotene/linoleic acid bleaching method, widely used in laboratories around the world. Since no high temperatures are required, the antioxidant capacity of thermo-sensitive extracts may be determined and qualitatively evaluated [29]. Antioxidant activity was determined from the ability of samples to inhibit β-carotene bleaching caused by free radicals generated during linoleic acid peroxidation. Antioxidant activity was classified as high (>70%), intermediate (40–70%) or low (<40%) levels of oxidation inhibition [30]. EE_FB displayed the highest level of antioxidant activity with a %AA of 70.5%. The intermediate group included EE_B, EE_L, EO_FB and BHT with %AA of 54.5, 41.7 and 58.2 %, respectively. Oils from berries and leaves exhibited low antioxidant activities, less than 40% with %AA value of 34.5 and 19.4% respectively.

Antioxidant activity of all EOs and EEs was organ-dependent. When organised in decreasing order of antioxidant activity, the ranking was: FB>B>L.

In the present study, the chemical composition and the antioxidant activity of EOs and EEs of myrtle berries, leaves and floral buds were evaluated. A strong variability in EOs and EEs composition depending on the plant organ was observed and seemingly is the origin of the variability of their potential antioxidant activities. Floral buds exhibited the highest antioxidant activity.

EO_FB was found to possess higher radical-scavenging and antioxidant activity. This difference could be attributed to the higher content in monoterpenes hydrocarbons. According to Tepe et al. [31], the former compounds present remarkable antioxidant activity due to the presence of strongly activated methylene groups. Moreover, the co-existence of linalool, eugenol and methyl eugenol in floral buds essential oil enhances the antioxidant activity due to their synergetic effects [32].

Concerning EEs, the antioxidant activity varied among the studied parts. It could be explained by the differences in polyphenolic compounds amounts. As previously reported [10,14], the polyphenolic content was strongly dependent on myrtle organs. Floral buds showed the highest antioxidant activity when analyzed by the two methods and the highest flavonoids content if compared to leaves and berries. This observation supports the hypothesis that flavonoids contribute directly to the antioxidant activity [33]. The relationship between the antioxidant activity and

polyphenolic compounds could be explained in several ways: the total polyphenolic fraction does not incorporate all the antioxidants, and synergetic interactions between the antioxidants in the mixture make the antioxidant activity not only depend on the concentration, but also on the structure and the nature of the antioxidants [34].

4. CONCLUSION

It is worthwhile to know the way the antioxidant activity varies depending on the organ type. This knowledge will impact the decision of choosing the most appropriate organ type and harvest timing to maximise antioxidant properties of myrtle extracts and essential oils. This may suggest as well new innovative uses in the sector of phytotherapy, pharmaceuticals, cosmetics and agro-food industry.

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