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Improvement of the Composition of Tunisian Myrtle Berries (*Myrtus communis* L.) Alcohol Extracts

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ABSTRACT: Different extracts from myrtle berries were obtained using alcohol−water mixtures as an extraction medium in the range of 60−90% (v/v) to study the extraction efficiency in the preparation of myrtle liqueur. Flavonoids and anthocyanins were identified by high-performance liquid chromatography (HPLC) coupled with electrospray mass spectrometry and quantified during the maceration period by HPLC coupled with ultraviolet/visible detection. The antioxidant activity was tested by the 2,2-diphenyl-1-picrylhydrazyl assay. Dry matter, pH, and color parameters (*L*, *a*, *b*) were also analyzed. At the end of the maceration period, EE80 showed better anthocyanins stability and the highest total antioxidant activity (87.5%). These results suggest that the use of ethanol 80% provides the extract with the best characteristics for liqueur preparation. The present study contributes significantly to increase the marketing appeal of myrtle berries.

KEYWORDS: *Myrtus communis* L. berries, ethanolic extracts, chemical composition, extraction medium

INTRODUCTION

Myrtus communis L. (commonly known as myrtle) is a stenomediterranean species belonging to the family of *Myrtaceae*. In Tunisia, the myrtle tree is found growing from Tabarka, Ain Draham, Sejenane, Zaghouan, and Bou Kornine to Cap-Bon (Zembra).[1] It is an evergreen shrub or a small tree that adapts to many kinds of soil.[2] Flowers are white, single, and very beautiful; fruits are 2−4 cm in diameter, mostly red to purples drupes when ripe, and very aromatic, while seeds are snail-shaped and covered by a thick coat.[3]

The plant finds several uses for culinary, cosmetic, and medicinal purposes. Fruits are very astringent and used as a condiment as a substitute for pepper and are considered a rich source of tannin.[4] Oils extracted by steam distillation of fruits are used both in flavor and in fragrance industries.[5] As a folk medicine, leaf and fruit decoctions or infusions of this plant are used as stomachic and hypoglycemic medicines, for cough and oral diseases, as an antibacterial, for constipation, appetizing, as an antihemorrhagic medicine, and externally for wound healing.[6−8]

The use of myrtle berries to make a liqueur is a very popular practice in Sardinia, and produced are two very famous liqueurs: The macerated fruits yield the Mirto Rosso, while the macerated leaves produce the Mirto Bianco. At present, the Sardinian production of myrtle liqueur has reached more than 3 million bottles per year, and the product has seen great success and has become one of the most typical Sardinian products exported abroad.[9]

Myrtle liqueur is an alcoholic beverage obtained by maceration of the plant material in aqueous ethanol for 40 days with subsequent addition of sugar and deionized water. The extraction procedure is of great importance for the product final quality. The selection of the extraction medium is determinant for the extraction of target compounds. The choice of the extraction medium should be such that it should extract the maximum of compounds of interest with a minimal amount of adjuncts and little degradation or alteration of their natural state.

In the present work, different extracts from Tunisian myrtle berries for the production of liqueur were obtained using alcohol−water mixtures as the extraction medium in the range of 60−90% (v/v) to select the most efficient medium. During the maceration period, we analyzed the chemical composition of the extracts. We mainly focused on the phenolic fractions, the major feature of this liqueur, since they strongly contribute to the color, taste, and texture of the product and because they are known to exhibit several health beneficial activities such as antioxidant, anti-inflammatory, antihepatotoxic, antitumor, and antimicrobial activities.[10,11]

MATERIALS AND METHODS

Chemicals. Standards of anthocyanins (delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside) and of flavonoids (myricetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, and myricetin) were purchased from Extrasynthese (France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma-Aldrich Chemie (Steinheim, Germany). All reagents were of analytical grade.

Plant Material. *M. communis* L. berries were collected from the region of Jabbalah in Ain-Draham (North West of Tunisia) in January 2007. Botanical identification of this species was carried out according to the Tunisian flora.[12] A voucher specimen has been kept in our unit for future reference. After the selection and removal of impurities, berries

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Table 1. Physical Characteristics of Myrtle Berries

properties	values
moisture content (%)	73.51 ± 1.14
length (mm)	10.20 ± 0.38
width (mm)	7.16 ± 0.51
weight (g % berries)	8.37 ± 0.75
pericarp yield in berries (%)	67.30 ± 0.58
no. of seeds per berry	8.43 ± 1.20

were packaged under vacuum and stored at −18 °C. Full details of fruit collection data are provided in Table 1.

Ethanolic Extracts (EEs) Preparation. In this work, EEs were prepared following a procedure originally proposed by Tubereso et al.[9] A 130 g amount of myrtle berries was put in dark bottles and left to step separately in 250 mL of different extraction media 60, 70, 80, and 90% of food-grade ethanol for 40 days.

Samples from different EEs were taken on days 10, 20, 30, and 40 of maceration and filtered through a 0.45 μm membrane before analysis. Each experiment was carried out in triplicate, and results were stated in means ± standard deviations.

Extracts Characterization. *pH and Dry Matter.* The pH was measured using a Consort P902 pH meter (Scientific Instrument). Dry matter was determined by drying 1 g of the alcoholic extract for 24 h at 103 ± 2 °C and weighting after it reached a constant temperature.[13]

Color Measurement. The extract color parameters were measured with a Lovibond PFX195 colorimeter based on the CIE $L^*a^*b^*$ coordinates. L^* describes lightness of the color, going from black ($L^* = 0$) to white ($L^* = 100$, perfect white); a^* takes a positive value for reddish colors and a negative value for the greenish ones, whereas b^* takes a positive value for yellowish colors and a negative value for the bluish ones. The sample was used without any dilution, in a 2 mm quartz cell.

High-Performance Liquid Chromatography−Ultraviolet (HPLC-UV) Analysis. Free anthocyanins and flavonoids were analyzed according to the slightly modified method of Tuberoso et al.[13] Analyses were carried out using an Agilent Technologies 1100 series liquid chromatograph coupled with a UV variable detector. A Simmetry C_{18} (250 mm × 4.6 mm, 5 μm, Waters) column was used for separation. The elution system consisted of mobile phase A, 0.2 M phosphoric acid, and mobile phase B, acetonitrile/0.2 M phosphoric acid (80:20, v/v). The flow rate was kept at 1 mL min^{-1}. The gradient program was as follows: $t = 0$ A:B (90:10; v/v), reaching 80:20 (v/v) in 15 min, then 60:40 (v/v) in 10 min, and finally 30:70 (v/v) in 20 min. The injection volume was 20 μL, and peaks were monitored at two specific wavelengths: 350 nm for flavonoids and 520 nm for anthocyanins.

HPLC-MS Analysis. HPLC-MS experiments were performed with an Esquire-LC ion trap LC/MS system with an ESI interface (Bruker Daltonics, Bremen, Germany) connected with an Agilent Technologies 1100 series HPLC system equipped with an UV−visible absorbance detector. The compounds were separated on a ZorbaxEclipse XDB-C_{18} (2.1 mm × 150 mm; 3.5 μm) column with the elution of two solvents: solvents A (aqueous 0.1% formic acid) and B (acetonitrile/0.1% formic acid). Separation was performed at 35 °C by the same gradient program used for HPLC-UV analysis at a flow rate of 0.2 mL min^{-1}. The injection volume was 5 μL.

Mass spectral measurements were recorded in the negative mode with the following ion optics: capillary voltage, 3 kV; spray voltage, 5 kV; and source temperature, 250 °C. Nitrogen was supplied at the flow of 60 (arbitrary units). Spectra were recorded in the full scan mode over the range m/z 100−1500, and the number of microscans was 5.

Quantitative Analysis. Quantitative analyses were assessed with the external standard method by integration of the absorbance in HPLC-UV against a calibration curve obtained from dilution series of a standard solution. Myricetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, myricetin, delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside were respectively used as external standards for flavonoids and anthocyanins using five concentrations (0, 5, 25, 50, 100, and 200 μg/mL) to construct a linear calibration curve with correlation coefficients (r^2) above 0.995 in all cases.

Antioxidant Activity. The antioxidant activity was measured for the extracts obtained after 40 days of maceration by the free radical DPPH assay.[14] A 0.1 mL amount of the extract was mixed with 2.9 mL of 0.1 mM DPPH in methanol and incubated in the dark at room temperature for 30 min; the absorbance (A) of the sample was measured at 515 nm using methanol as a blank. All tests were performed in triplicate. The total antioxidant activity (TAA) was expressed in terms of percentage activity as calculated with the following equation:

$$\%TAA = 100 \times [(A_C - A_S)/A_C]$$

where A_C is the absorbance of the control and A_S is the absorbance of the tested sample.

Statistical Analysis. Differences were tested for significance by the analysis of variance procedure (Statgraphics Centurion XVI) using a significance level of $p \leq 0.05$. Pearson's correlation coefficients were calculated at 5% probability using Student's t test for all variables.

Table 2. Evolution of pH and Dry Matter (%) and CIE $L^*a^*b^*$ Coordinates during Maceration

	pH	dry matter (%)	L^*	a^*	b^*
			EE60		
10	5.58 ± 0.09	5.89 ± 0.05	41.7 ± 1.2	40.1 ± 2.5	−10.8 ± 1.2
20	5.48 ± 0.15	6.15 ± 0.07	37.8 ± 0.6	41.2 ± 3.7	−7.6 ± 0.7
30	5.35 ± 0.13	6.74 ± 0.02	33.9 ± 2.4	43.5 ± 1.9	−2.3 ± 0.2
40	5.46 ± 0.04	6.45 ± 0.10	31.6 ± 3.5	42.3 ± 1.5	−1.6 ± 0.1
			EE70		
10	5.67 ± 0.10	5.84 ± 0.06	43.1 ± 4.1	41.8 ± 1.6	−11.3 ± 0.6
20	5.56 ± 0.08	6.04 ± 0.03	36.2 ± 1.8	44.6 ± 4.3	−8.5 ± 1.7
30	5.50 ± 0.05	6.58 ± 0.04	35.5 ± 2.7	44.9 ± 3.9	−4.1 ± 0.4
40	5.51 ± 0.13	6.27 ± 0.07	32.8 ± 1.3	43.7 ± 2.7	−2.7 ± 0.7
			EE80		
10	5.72 ± 0.12	5.43 ± 0.02	44.0 ± 1.7	42.7 ± 3.4	−12.4 ± 0.8
20	5.57 ± 0.11	5.92 ± 0.10	39.7 ± 3.1	43.8 ± 2.8	−9.7 ± 0.5
30	5.52 ± 0.02	6.51 ± 0.10	37.6 ± 1.8	45.1 ± 2.1	−5.3 ± 0.9
40	5.53 ± 0.07	6.29 ± 0.03	34.2 ± 2.9	44.1 ± 1.3	−1.4 ± 0.3
			EE90		
10	5.70 ± 0.07	5.17 ± 0.08	46.4 ± 2.3	41.9 ± 1.8	−12.9 ± 1.3
20	5.65 ± 0.10	5.31 ± 0.09	41.8 ± 3.5	44.6 ± 4.1	−10.2 ± 1.1
30	5.56 ± 0.06	5.93 ± 0.04	38.4 ± 1.6	43.5 ± 3.7	−4.9 ± 0.5
40	5.56 ± 0.12	5.64 ± 0.06	35.1 ± 1.1	43.8 ± 3.0	−2.5 ± 0.6

RESULTS AND DISCUSSION

Myrtle Berries Characteristics. Myrtle berries used in this study were collected at the time of ripeness when they are dark blue pigmented according to Traveset et al.[15] The physical properties of myrtle berries determined at 73.51% moisture content are given in Table 1. The average length, width, and weight were measured as 10.20 mm, 7.16 mm, and 8.37 g % berries, respectively. The fruit is constituted of pericarp and approximately 8 seeds, which represent 67.30 and 32.70% of the whole ripe fruit. Higher values were observed with Turkish myrtle fruits reported

by Aydin and Özcan,[16] which offered a length of 13.7 mm, a width of 8.1 mm, a weight of 132.6 g % fruits, and a moisture content of 74.44%. These variations could be related to several differences such as geography, environment, harvest period, berry maturity, and variety type. The determination of physical properties of myrtle berries is believed to be helping the processing technology.

pH, Dry Matter, and Color Coordinates. Table 2 shows pH, dry matter, and color coordinates evolution in extracts during the maceration period.

The pH values of all samples fell around 5.5. At the end of the maceration process, EE90 showed the highest value (5.56), while EE60 showed the lowest (5.46). The pH was not affected by the alcohol content in the extraction medium during maceration.

During the maceration process, changes in dry matter content were observed. These changes are mainly due to the diffusion of substances coming from the myrtle fruit to the surrounding medium. The highest values of dry matter content were obtained on the 30th day of maceration. After, a slow decrease was noticed. Differences depending on the extraction medium were found. EE60 showed the highest dry matter values throughout the maceration period. This behavior might indicate extraction of higher amounts of concomitant substances.

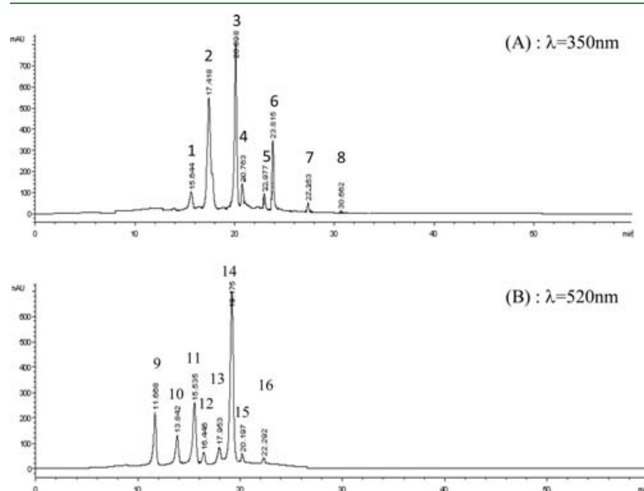

Figure 1. HPLC-UV chromatograms of major compounds in myrtle extracts at (A) 350 and (B) 520 nm. The extract was obtained with 70% ethanol after 40 days of maceration.

The lightness values decreased during maceration for all samples with higher values observed for EE90. After 40 days of maceration, values ranged between 31.6 and 35.1. The lightness decrease could be attributed to oxidation, which was probably caused mainly by the increased presence of oxygen in the space at the top of the bottles.

The a^* values increased during the maceration and fell between 42.3 and 44.1 at the end of maceration process, indicating a high red prevalence. However, the color differences were down to 3 CIE$L^*a^*b^*$ units showing that they cannot be visually discriminated.[17] The parameter b^* increased throughout maceration and reached values close to zero, indicating the presence of reddish tone for these extracts.

HPLC-UV Analyses. *Compounds Identification.* The chromatographic analyses of the EEs from myrtle berries led to the separation of eight flavonoids (Figure 1A) at 350 nm and eight anthocyanins (Figure 1B) at 520 nm.

Among these compounds, myricetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, and myricetin with peaks at 350 nm and delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside with peaks at 520 nm were identified by comparison of their HPLC retention times and UV visible absorption with the authentic compounds.

The lack of commercially available standards entailed the use of other methodologies, permitting the identification of individual components of a mixture. Numerous analytical techniques including HPLC-UV, HPLC-MS, and HPLC-MS/MS were applied for the separation and detection of phenolic compounds in wine samples.[18−21] In our study, HPLC-MS was selected as an analytical method for the identification of compounds in extracts

Table 3. Retention Time and MS Fragmentation Data on the Negative Ionization Mode of Major Compounds Detected at 350 and 520 nm in Myrtle Extracts

peaks[a]	λ (nm)	R_t[b](min)	$[M - H]^{-c}$	identification	bases[d]
1	350	15.644	479	myricetin-3-*O*-galactoside	MS, UV
2	350	17.418	463	myricetin-3-*O*-rhamnoside	R, MS, UV
3	350	20.090	449	myricetin-3-*O*-arabinoside	MS, UV
4	350	20.783	463	quercetin-3-*O*-glucoside	R, MS, UV
5	350	22,977	447	quercetin-3-*O*-rhamnoside	MS, UV
6	350	23.815	317	myricetin	R, MS, UV
7	350	27.353	301	quercetin	MS, UV
8	350	30.662	285	kaempferol	MS, UV
9	520	11668	463	delphinidin-3-*O*-glucoside	R, MS, UV
10	520	13.842	447	cyanidin-3-*O*-glucoside	MS, UV
11	520	15.535	477	petunidin-3-*O*-glucoside	R, MS, UV
12	520	16.446	433	delphinidin-3-*O*-arabinoside	MS, UV
13	520	17.953	461	peonidin-3-*O*-glucoside	MS, UV
14	520	19.175	491	malvidin-3-*O*-glucoside	R, MS, UV
15	520	20.197	447	petunidin-3-*O*-arabinoside	MS, UV
16	520	22.292	461	malvidin-3-*O*-arabinoside	MS, UV

[a] Peak numbers refer to HPLC analysis in Figure 1A,B. [b] R_t, retention time, refers to HPLC analysis in Figure 1A,B. [c] $[M - H]^-$, molecular ion in the negative ionization mode. [d] R, reference compounds; MS, mass spectrum; and UV, ultraviolet spectrum.

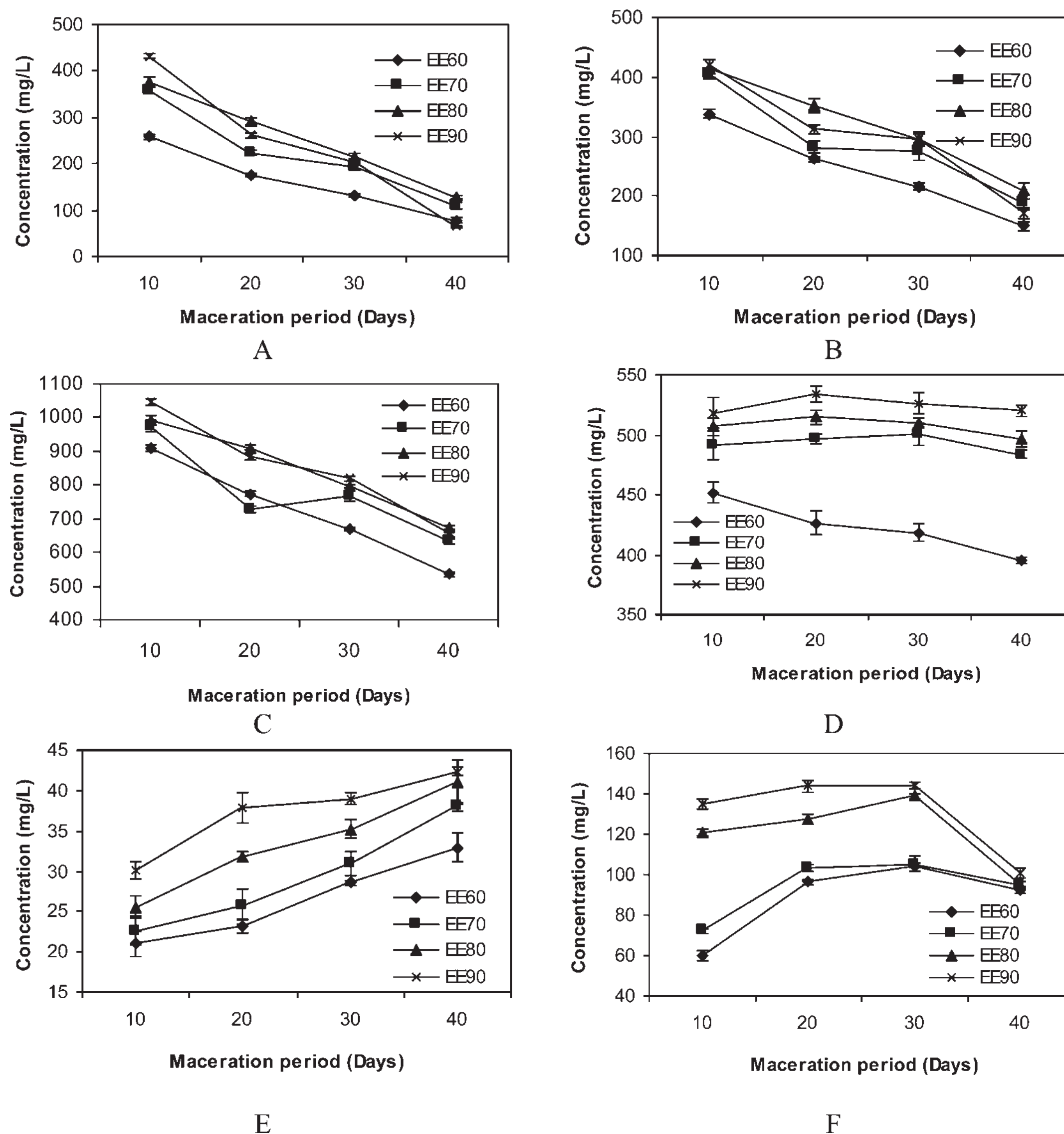

Figure 2. Evolution of (A) delphinidin-3-*O*-glucoside, (B) petunidin-3-*O*-glucoside, (C) malvidin-3-*O*-glucoside, (D) myricetin-3-*O*-rhamnoside, (E) quercetin-3-*O*-glucoside, and (F) myricetin during the maceration period.

because of its high selectivity and sensitivity.[22] According to the chromatographic separation method used for HPLC-MS analysis, anthocyanins and flavonoids in the extracts and their MS data are presented in Table 3.

Myrtle berries extracts used for the preparation of liqueur are reported to contain delphinidin, petunidin, malvidin, peonidin, and cyaniding-3-mono- and 3,5-diglucosides.[23] Recently, Barboni et al.[24] have studied the polyphenolic composition of *M. communis* berries extracts. Fourteen components, using HPLC-diode array detection (DAD) and LC-MS/MS, were identified as follows: two phenolic acids (gallic acid, ellagic acid), four flavanols [(+)-catechin, (−)-epicatechin-3-*O*-gallate, (−)-epigallocatechin, (−)-epigallocatechin-3-*O*-gallate], five flavonol glycosides (myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside, myricetin-3-*O*-arabinoside, quercetin-3-*O*-glucoside, and quercetin-3-*O*-rhamnoside) and three flavonols (myricetin, quercetin, and kaempferol). The presence of quercetin and kaempferol was reported for the first time in berry alcoholic extracts from *M. communis*. In our study, these two compounds were identified.

Quantitative Analysis. All anthocyanins and flavonoids concerned by the quantitative analyses were found to be present at high concentrations, allowing their determination by direct injection of samples. Figure 2 shows the evolution of delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, myricetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, and myricetin during the maceration period in different extracts.

It is obvious that the identified anthocyanins evolved similarly in different extracts during the maceration period (Figure 2A−C), although slight differences in their concentrations can be observed. After 10 days of maceration, EE90 exhibited higher amounts of anthocyanins than did other extracts. The difference was due to the large contribution of ethanol percentage. In fact, an increasing water proportion in the extraction medium significantly reduced the amount of phenolic compounds extracted.[25−27]

During the maceration period, we noticed a reduction in the concentrations of the identified compounds in all extracts. Thus, the content of malvidin-3-*O*-glucoside, the major constituent of the extracts, decreased with a loss of 30−40% in 40 days. A better

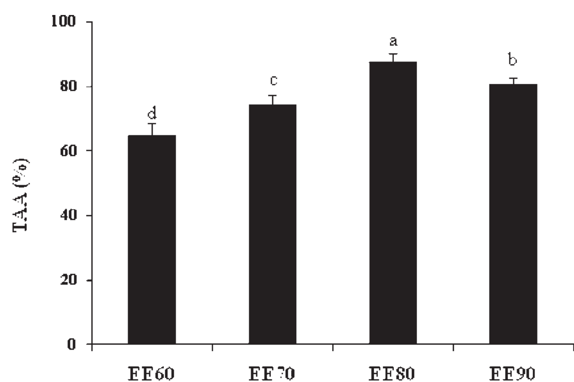

Figure 3. Total antioxidant activities of myrtle extracts after 40 days of maceration measured by DPPH assay. Data are expressed as means ± SDs of three replicates. Values followed by the different small letter share significant differences at 5%.

stability was observed in EE80. At the end of the maceration period, concentrations on delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside of EE80 were 126.70, 206.60, and 672.90 mg/L, respectively.

The decrease in free anthocyanins concentrations is probably due to their degradation or combination with other compounds to give more stable polymeric pigments.[28] As previously described by others authors, the decrease of anthocyanins could be due to three different mechanisms: the formation of stable polymers by copigmentation with flavan-3-ols[29] or flavonols,[30] acetaldehyde-mediated condensations,[31] and/or the formation of copolymers with the quinine of the caftaric acid and anthocyanins.[32] There was no significant difference in anthocyanins concentrations between the studied extracts.

The concentrations on delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside found in this work are higher than those previously determined by Tuberoso et al.[13] at the end of the maceration period. These authors compared the anthocyanins contents of myrtle extracts from five different selections. The concentrations varied from 19 to 86.3 mg/L for delphinidin-3-*O*-glucoside, 22.1 to 101 mg/L for petunidin-3-*O*-glucoside, and 58.4 to 258 mg/L for malvidin-3-*O*-glucoside.

As regarding flavonoids (Figure 2D−F), they showed a different evolution during the maceration period. Indeed, myricetin-3-*O*-rhamnoside was stable and showed comparable values during the maceration period, quercetin-3-*O*-glucoside softly increased for all extracts, and myricetin increased during the first 30 days and later decreased.

EE90 showed the highest amounts in flavonoids during the maceration period. At the first sampling, its content on myricetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, and myricetin was, respectively, about 518.3, 30.2, and 135.2 mg/L. At the end of the maceration period, we found contents on myricetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, and myricetin of, respectively, about 520.1, 42.3, and 100.5 mg/L.

The lowest concentrations of flavonoids were found with EE60. The concentrations obtained in EE70 and EE80 were intermediate with concentrations of EE60 and EE90. Among the quantified flavonoids, myricetin-3-*O*-rhamnoside and myricetin were significantly affected by the extraction medium.

The oscillation in the flavonoids concentrations is partly due to mutual polymerization−depolymerization processes accompanied by combination with some anthocyanins to give more stable pigments and partly due to the formation of procyanidins.[33] The dry matter content did not correlate with the free anthocyanins and flavonoids contents in extracts. The addition of water to ethanol improved the extraction rate, but too high of a water content brought an increased concomitant extraction of other compounds such as proteins, peptides, polysaccharides, etc.[34]

Table 4. Pearson's Correlation Coefficients (r) between Different Study Parameters of Myrtle Berries Extracts

	Del-3glu	Pet-3glu	Mal-3glu	Myr-3rha	Quer-3gly	Myr
pH	0.80[b]	0.78[b]	0.80[b]	0.65	0.08	0.22
dry matter	−0.64[a]	−0.61[a]	0.69[a]	−0.57	0.03	−0.29
L^*	0.94[b]	0.94[b]	0.98[b]	0.45	−0.43	0.24
a^*	−0.28	−0.29	−0.32	0.49	0.62	0.54
b^*	−0.93[b]	−0.93[b]	−0.93[b]	−0.35	0.58	−0.12

[a] Significant at $p < 0.05$. [b] Highly significant at $p < 0.05$.

Antioxidant Activity. The total antioxidant activities of EEs were evaluated by DPPH assay (Figure 3). The method is based on the reduction of the stable radical, DPPH, and the formation a nonradical form in the presence of a hydrogen-donating antioxidant. The extracts showed an antioxidant activity by reducing DPPH to the yellow-colored diphenylpicrylhydrazine derivates.

Many studies showed that essential oils and extracts from different *M. communis* L. organs have appreciable antioxidant activity.[35−37] In the present study, the antioxidant activity varied significantly ($p < 0.05$) between different extracts. The maximum value of antioxidant efficiency after 40 days of maceration was observed in EE80 (87.5%), whereas the minimum was found in EE60 (65.0%).

Differences in the total antioxidant activities among myrtle extracts could be attributed to their differences in phenolic compound amounts and structures. The antioxidative properties of plant extracts are correlated not only with the total amount of antioxidants but also with the presence of selected compounds.[38] The TAA of the studied extracts can be interpreted as the combined action of different endogenous antioxidants and the newly formed compounds during the maceration. EE80 showed the highest TAA and probably the best combination of phenolic compounds.

With the objective of determining a possible relationship between the study parameters, Pearson's correlation coefficients were calculated (Table 4). A positive and significant correlation was found between pH-anthocyanins ($r = 0.78−0.80$) and lightness L^*-anthocyanins ($r = 0.94−0.98$). Higher values of lightness indicate higher amounts of anthocyanins, the main compounds responsible of the final liqueur quality. Dry matter and anthocyanins were negatively and significantly correlated ($r = −0.61$ to $−0.69$). This supports the hypothesis that the increase of the alcohol percentage in the extraction medium led to a better extraction of phenolic compounds. There was also a negative and significant correlation of b^* and anthocyanins ($r = −0.93$); this is due to the fact that b^* takes an initially negative value and increases during the maceration. b^* can be regarded as the characteristic parameter of myrtle extracts maturity and possibly values close to zero, indicating a reddish tone, at the end of the maceration could become a marker of the maturity of myrtle extracts.

To the best of our knowledge, this is the first study on the influence of the alcohol concentration on the phenolic composition and antioxidant properties of myrtle extracts used to make a liqueur. The obtained results revealed that the use of alcohol−water

mixtures as extraction medium in the range 60−90% (v/v) gave extracts with same chemical characteristics. There was no significant difference between the extracts, although the increase of water content in the extraction medium enhances the coextraction of concomitants leading to the reduction of sensory and antioxidant properties of extracts used for the production of myrtle liqueur. The evaluation of the antioxidant activity, as a free-radical scavenging ability, showed that EE80 had the highest TAA among all extracts. On the basis of all the above, it can be concluded that ethanol:water (80:20) provides the extract with the best characteristics for liqueur preparation.

Furthermore, the interesting antioxidant properties of myrtle extracts might enhance their use in the improvement of nutraceutical characteristics of foods and their preservation. These extracts could be used for the production of natural colorants and antioxidants, providing some economic benefits and added value to these berries.

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