Journal of Food and Drug Analysis

Volume 24 | Issue 2 Article 18

Characterization of myrtle seed (Myrtus communis var. baetica) as a source of lipids, phenolics, and antioxidant activities

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Characterization of myrtle seed (*Myrtus communis* var. *baetica*) as a source of lipids, phenolics, and antioxidant activities

Wissem Aidi Wannes[*], *Brahim Marzouk*

Laboratory of Bioactive Substances, Biotechnologic Center Borj-Cedria Technopark, Hammam-Lif, Tunisia

ARTICLE INFO

Article history:
Received 8 August 2015
Received in revised form
16 October 2015
Accepted 19 November 2015
Available online 8 January 2016

Keywords:
antioxidant activity
fatty acids
lipids
Myrtus communis var. *baetica*
oil
polyphenols
seed

ABSTRACT

This study aimed to characterize the chemical composition and antioxidant activity of the oil and the methanolic extract of *Myrtus communis* var. *baetica* seed. The oil yield of myrtle seed was 8.90%, with the amount of neutral lipid, especially triacylglycerol, being the highest, followed by phospholipids and glycolipids. Total lipids and all lipid classes were rich in linoleic acid. The content of total phenols, flavonoids, tannins, and proanthocyanidins of the methanolic extract and the oil from myrtle seed was determined using spectrophotometric methods. Antioxidant activities of the oil and the methanolic extract from myrtle seed were evaluated using 1,1-diphenyl-2-picrylhydrazyl radical scavenging, β-carotene–linoleic acid bleaching, and reducing power and metal chelating activity assays. In all tests, the methanolic extract of myrtle seed showed better antioxidant activity than oil. This investigation could suggest the use of myrtle seed in food, industrial, and biomedical applications for its potential metabolites and antioxidant abilities.

1. Introduction

Myrtus communis L. is an aromatic medicinal species that belongs to the family Myrtaceae. In Tunisia, the genus *Myrtus* is represented by only one species, *M. communis*, which grows wild in the coastal areas, the internal hills, and the forest areas of North Tunisia. Two myrtle varieties, namely, *M. communis* var. *italica* L. and *M. communis* var. *baetica* L, having the same vegetative characters have been described previously [1]. The morphological difference between the two varieties is the larger size of *baetica* fruits and leaves.

Myrtle is a pleasant annual shrub with dark blue ripe berries, which have a long history of application in the perfume, cosmetic, food, and pharmaceutical industries [2]. In addition, these berries are widely used in industrial formulation of sweet liqueur [3]. Myrtle seed produced as a by-product

* *Corresponding author*. Laboratory of Bioactive Substances, Biotechnologic Center Borj-Cedria Technopark, B.P. 901, 2050 Hammam-Lif, Tunisia.
E-mail address: aidiwissem@yahoo.fr (W.A. Wannes).

http://dx.doi.org/10.1016/j.jfda.2015.11.001

during the processing of sweet liqueur constitutes about 36.5% of the whole berry weight [4].

To date, most studies in this area have focused on extraction of essential oils [5–15], lipids [4,16], phenolic compounds, and on utilizing antioxidant activities of myrtle fruit [17–23]. By contrast, it is hard to find any data in the literature about myrtle seed except our two previous studies [4,22].

In fact, it has been shown that seeds of *M. communis* var. *italica* could be a good source of antioxidants in association with bioactive compounds, particularly polyphenols [22]. There is an increasing interest in using naturally occurring antioxidants in food, cosmetic, and pharmaceutical products to replace synthetic antioxidants, which are reported to have carcinogenic effects [24,25].

In a previous study, we also analyzed the lipid composition of myrtle seed oil [4]. Myrtle (*M. communis* var. *italica*) seeds have an appreciable oil yield of about 12%; additionally, they are rich sources of polyunsaturated linoleic acid [4]. In general, oils rich in linoleic acid are used as a raw material in the manufacture of conjugated linoleic acid [26], a novel therapeutic nutrient with promising antioxidant and antitumor properties [27].

Utilizing the concept of myrtle seed valorization, this study aimed to characterize the seed of *M. communis* var. *baetica* regarding its lipid profile, phenolic content, and antioxidant activity.

2. Materials and methods

2.1. Plant material

Seeds of *M. communis* var. *baetica* L. were collected from Jbal Er-Rimel of Bizerte region. The plants were identified by Professor A. Smaoui (Biotechnologic Center in Borj-Cedria Technopark, Tunisia). A voucher specimen (Mcb06006) has been deposited in our herbarium.

2.2. Continuous extraction of oil

Myrtle seed was ground in an electric grinder. About 20 g of each ground sample was added to an extraction cartridge made up of cellulose and placed in a Soxhlet extractor. Petroleum ether was used as the extraction solvent. After 8 hours of extraction, the extracted solution was evaporated under vacuum, weighed, and the oil yield was determined.

2.3. Total lipid extraction

Triplicate subsamples (1 g) were extracted using the method suggested by Bligh and Dyer [28], but with some modifications. The seed samples were fixed in boiling water for 5 minutes and then ground manually in a China mortar using a mixture of chloroform/methanol/hexane (3:2:1, v/v/v). After washing with water of fixation and decantation for 24 hours at 4°C, the organic phase containing total lipids (TLs) was recovered and dried under a nitrogen stream. Finally, the residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) and stored at −20°C for further analyses.

2.4. Separation of lipid classes by thin-layer chromatography

Lipid classes were separated by thin-layer chromatography using 20 cm × 20 cm × 0.25 mm silica gel plates (G60, Merck, Darmstadt, Germany). Neutral lipids (NLs) were separated by adopting the method described by Mangold [29], using a development system composed of petroleum ether–diethyl ether–acetic acid (70:30:0.4; v/v/v). Polar lipids were separated using a mobile phase mixture consisting of chloroform–acetone–methanol–acetic acid–water (50:20:10:10:5; v/v/v/v) as described by Lepage [30]. Lipid spots were detected after a brief exposure to iodine and each spot was identified by co-chromatography of pure lipid standards.

2.5. Fatty acid methylation and analysis

Total fatty acids (TFAs) and those of lipid classes were transformed into their corresponding methyl esters as described by Cecch et al [31]. Transmethylation was accomplished by the addition of 2 mL of hexane, 0.5 mL of 3% sodium methylate, a known amount of heptadecanoic acid methyl ester (C17:0) used as the internal standard, 0.2 mL of 1N H_2SO_4, and 1.5 mL of 10% sodium chloride. The hexanic phase that contains fatty acid methyl esters (FAMEs) was recovered and its volume was reduced using a stream of nitrogen prior to analysis. FAME analysis was carried out by gas chromatography (GC) using a Hewlett-Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector and an electronic pressure control injector. For GC analysis, an HP-INNOwax capillary column (polyethylene glycol: 30 m × 0.25 mm i.d., 0.25-μm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA) was used; the flow of the carrier gas (N_2, U) was 1.6 mL/min and the split ratio was 60:1. Analyses were performed using an oven temperature of 150°C for 1 minute, followed by an increase from 150°C to 200°C at a rate of 15°C/min, and then from 200°C to 225°C at a rate of 2°C/min, and finally held at this temperature for an additional 2-minute period. The detector and injector temperatures were set at 275°C and 250°C, respectively. FAMEs were identified by comparison of their retention times with those of pure reference standards. The gas chromatograph was connected to an HP ChemStation (Rev.A.0401) software for peak area and fatty acid percentage calculation.

2.6. Total phenolic content

The total phenolic contents were assayed using the Folin–Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al [32]. The dried extract was redissolved in methanol. An aliquot (0.125 mL) of a suitably diluted methanolic solution was added to 0.5 mL of deionized water and 0.125 mL of the Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before adding 1.25 mL of 7% Na_2CO_3 solution. The solution was then adjusted by adding deionized water to make up a final volume of 3 mL and mixed thoroughly. After incubation for 90 minutes at 23°C, the absorbance versus a prepared blank was read at 760 nm. The total extractable phenolic contents of methanolic extract and oil (three replicates per treatment) were expressed

as milligram gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50–400 mg/mL ($R^2 = 0.99$). All samples were performed in triplicates.

2.7. Total tannin content

The total tannin content was determined using the Folin–Ciocalteu method by calculating the difference in total content following the adsorption of tannin onto hide powder/polyvinylpyrrolidone (PVPP) [33]. In brief, the seed extract was homogenized with the adsorbent material and the mixture was stirred for 30–60 minutes. The preparation obtained was stored for 1–2 hours at 4°C to allow the formation of the tannin–PVPP complex. The pH was then acidified (pH 3) in case of using PVPP. Following centrifugation at 4000 rpm for 15 minutes, no adsorbed phenolics in the supernatant were determined by the Folin–Ciocalteu procedure as described earlier. Calculated values were subtracted from total polyphenolic contents and the difference was expressed as mg gallic acid 150 equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50–400 mg/mL ($R^2 = 0.99$).

2.8. Total flavonoid content

The total flavonoid content was measured according to the procedure reported by Dewanto et al [32]; 250 mL of appropriately diluted methanolic seed extract was mixed with 75 mL $NaNO_2$ (5%). After 6 minutes, 150 mL of 10% $AlCl_3$ and 500 mL of NaOH (1M) were added to this mixture. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance versus a prepared blank was read at 510 nm. The total flavonoid contents of methanolic extract and oil (3 replicates/treatment) were expressed as milligram catechin equivalents per gram (mg CE/g) through a calibration curve with catechin. The calibration curve range was 50–500 mg/mL.

2.9. Condensed tannin content

In the presence of concentrated H_2SO_4, condensed tannins transform into anthocyanidols due to their reaction with vanillin [34]. From the methanolic extract, 50 mL was appropriately diluted and was mixed with 3 mL of 4% methanol–vanillin solution and 1.5 mL of H_2SO_4. After 15 minutes, the absorbance was measured at 500 nm. Condensed tannin contents of the methanolic extract and oil (three replicates per treatment) were expressed as milligram catechin equivalents per gram (mg CE/g) through a calibration curve with catechin. The calibration curve range was 50–600 mg/mL.

2.10. 1,1-Diphenyl-2-picrylhydrazyl assay

The electron donation ability of the obtained extract and oil was measured by bleaching the purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method suggested by Hanato et al [35]. Approximately 1 mL of various concentrations of seed extract and oil (mg/mL) prepared in methanol were added to 0.5 mL of a 0.2 mmol/L DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 minutes. The absorbance of the resulting solution was then measured at 517 nm after 30 minutes. The antiradical activity (3 replicates/treatment) was expressed as IC_{50} (mg/mL), the concentration required to cause a 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect}(\%) = [(A_0 - A_1)A_0] \times 100$$

where:

A_0 = absorbance of the control at 30 minutes; and

A_1 = absorbance of the sample at 30 minutes.

Butylated hydroxytoluene (BHT) was used as a positive control. All tests were carried out in triplicate.

2.11. β-Carotene bleaching test

The β-carotene bleaching method is based on the loss of yellow color of β-carotene because of its reaction with radicals formed by oxidation of linoleic acid in an emulsion. The rate of β-carotene bleaching is slowed down in the presence of antioxidants [36]. A modification of the method described by Koleva et al [37] was used for the bleaching test. 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 ultrapure water was added. The emulsion was then shaken vigorously. Reference compounds [BHT and butylated hydroxyanisole (BHA)], seed extract and oil were prepared in methanol. The emulsion (3 mL) was added to a tube containing 0.2 mL of various concentrations of seed oil and extract (mg/mL). The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50°C for 120 minutes, when the absorbance was measured again. BHT and BHA were used as positive control. In the negative control, the extract or oil was substituted with an equal volume of methanol. The antioxidant activity (%) of these samples was evaluated in terms of bleaching of the β-carotene using the following formula:

$$\%\ \text{Inhibition} = [(A_t - C_t)\cdot(C_0 - C_t)] \times 100$$

where:

A_t and C_t = absorbance values measured for the test sample and control, respectively, after incubation for 120 minutes; and

C_0 = absorbance value for the control measured at zero time during the incubation. The results are expressed as IC_{50} values (mg/mL), the concentration required to cause a 50% β-carotene bleaching inhibition. Tests were carried out in triplicate.

2.12. Chelating effect on ferrous ions

The chelating activity of the sample (extract or oil) was determined by the ferrozine assay. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted due to which the red color of the complex is decreased. Measurement

of the rate of color reduction therefore allows for estimating the chelating activity of the coexisting chelator [38]. The ferrous ion chelating activity of seed extract and oil was assessed as described by Zhao et al [39]. Different concentrations of seed extract and oil (mg/mL) prepared in methanol were added to 0.05 mL of $FeCl_2.4H_2O$ solution (2mM) and incubated at room temperature for 5 minutes. Then, the reaction was initiated by adding 0.1 mL of ferrozine (5mM) and the mixture was adjusted to 3 mL by adding deionized water. The mixture was shaken vigorously and left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the following formula:

$$\text{Metal chelating effect}(\%) = [(A_0 - A_1)/A_0] \times 100$$

where:

A_0 = absorbance of the ferrozine–Fe^{2+} complex; and

A_1 = absorbance of the test compound.

Results were expressed as IC_{50}, which is the efficient concentration corresponding to 50% of ferrous iron chelation. EDTA was used as a positive control and all tests were carried out in triplicate.

2.13. Reducing power

In this assay, the yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution reduces the Fe^{3+}–ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by the measurement of the absorbance at 700 nm [40]. The method reported by Oyaizu [41] was used to assess the reducing power of seed extract and oil: 1 mL of various concentrations of seed extract and oil (mg/mL) in methanol were mixed with 2.5 mL of a 0.2M sodium phosphate buffer (pH = 6.6, prepared from 62.5 mL of a 0.2M Na_2HPO_4 and 37.5 mL of a 0.2M $NaH_2PO_4H_2O$) and 2.5 mL of 1% $K_3Fe(CN)_6$ and incubated in a water bath at 50°C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture that was centrifuged at 650*g* for 10 minutes. The supernatant (2.5 mL) was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the blue-green color was measured at 700 nm. The half-maximal effective concentration (EC_{50}) value (mg/mL) is the sample concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against sample concentration. Ascorbic acid was used as a positive control. Tests were carried out in triplicate.

2.14. Statistical analyses

All extractions and determinations were conducted in triplicates and results were expressed based on dry weight (DW). Data are expressed as mean ± standard deviation. The means were compared by the one-way and multivariate analysis of variance followed by Duncan's multiple-range tests. The differences between individual means were deemed to be significant at $p < 0.05$. All analyses were performed with the STATISTICA v 5.1 software [42].

3. Results and discussion

3.1. Levels of different lipid classes

The oil of myrtle seed constituted 8.90% of dry matter weight. Chromatographic procedures were used to obtain the different lipid classes of myrtle seed oil (Table 1). TLs were composed of NL, phospholipid (PL), and galactolipid (GL) fractions. The level of NL was the highest (93.68% of TL), followed by PL (3.17% of TL) and GL (3.12% of TL). These results were similar to those reported for oleaginous seeds (*Guizotia abyssinica* [43], *Cuminum cyminum* [44], and *Pistacia vera* [45]).

As can be seen in Table 1, the NL revealed the presence of four fractions. The results also showed that monoacylglycerol, diacylglycerol, and free fatty acids were weakly represented in myrtle seed. Triacylglycerol constituted the main NL, which accounted for 91.71% of TLs. The polar lipids of myrtle seed were divided into PL and GL. PL contained five classes, namely, phosphatidic acid (1.28% of TL), phosphatidylcholine (0.62% of TL), phosphatidylinositol (0.58% of TL), phosphatidylglycerol (0.39% of TL), and phosphatidylethanolamine (0.30% of TL). GL referred to monogalactosyldiacylglycerol (1.60% of TL) and digalactosyldiacylglycerol (1.52% of TL). The lipid profile of *M. communis* var. *baetica* seed was similar to that reported for *M. communis* var. *italica* [4].

3.2. Fatty acid composition of myrtle seed oil and its lipid classes

Fatty acid compositions of TL and lipid classes (NL, GL, and PL) are presented in Table 2. Because the TL pool was essentially made up of NL fraction, the fatty acid compositions of these two samples were very similar. It was also observed that all lipid fractions contained identical fatty acid components but with different proportions. They were richer in polyunsaturated fatty acids (PUFAs), essentially due to higher proportions of linoleic acid with 77.88% of TFA in TL, 84.67% of TFA in NL, 58.12% of TFA in GL, and 62.13% of TFA in PL. The proportion of polyunsaturated linolenic acid in GL (4.57% of TFA) and PL (3.26% of TFA) was higher than that in TL (0.34% of TFA) and NL (0.32% of TFA). Monounsaturated fatty acids (MUFAs) were represented by oleic acid, which constituted a higher proportion in GL (11.24% of TFA) and PL (9.83% of TFA) than in TL (7.73% of TFA) and NL (4.31% of TFA). The two other MUFAs, palmitoleic (C16:1 *n*–7) and gadoleic (C20:1 *n*–9) acids, were weakly represented in all lipid fractions. Saturated fatty acids (SFAs) were characterized by high levels of palmitic acid in all samples (16.44% of TFA in GL, 16.42% of TFA in PL, 10.17% of TFA in TL, and 8.26% of TFA in NL). The proportion of stearic acid in NL (0.65% of TFA) was lower than in TL (3.13% of TFA), PL (4.65% of TFA), and GL (4.76% of TFA). Lauric (C12:0), myristic (C14:0), and arachidic (C20:0) acids had a minimal presence in myrtle seed oil.

The ratio of SFA to PUFA (SFA/PUFA) was 0.18 in TL, 0.12 in NL, 0.40 in GL, and 0.37 in PL. This ratio was lower in neutral fraction than in polar fraction (GL and PL), which was due to the higher levels of SFAs (especially palmitic and stearic acids) in GL and PL than in NL. From our results, it could be concluded that the fatty acid profile of myrtle seed oil

Table 1 – Lipid composition of *Myrtus communis* var. *baetica* seed.

Lipid classes	Neutral lipid (% of TL)	Phospholipid (% of TL)	Galactolipid (% of TL)
Monoacylglycerol	0.96 ± 0.01	—	—
Diacylglycerol	0.60 ± 0.12	—	—
Free fatty acids	0.41 ± 0.11	—	—
Triacylglycerol	91.71 ± 1.71	—	—
Phosphatidylinositol	—	0.58 ± 0.12	—
Phosphatidylcholine	—	0.62 ± 0.11	—
Phosphatidylglycerol	—	0.39 ± 0.08	—
Phosphatidylethanolamine	—	0.30 ± 0.12	—
Phosphatidic acid	—	1.28 ± 0.73	—
Monogalactosyldiacylglycerol	—	—	1.60 ± 1.01
Digalactosyldiacylglycerol	—	—	1.52 ± 0.09
Total (% of TL)	93.68 ± 0.22 [a]	3.17 ± 0.25 [b]	3.12 ± 0.25 [b]

[a,b] Values (mean ± standard deviation, $n = 3$) in the same column with different superscripted letters are significantly different at $p < 0.05$ (Duncan test).
TL = total lipid.

presented the lipids as a good source of the nutritionally essential PUFA. There is an increasing interest in the oils that contain PUFA and a growing literature illustrated the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes, and other diseases [46]. The fatty acid composition and the high proportions of PUFA in all lipid fractions could be making the myrtle seed lipids important for nutritional and industrial applications.

3.3. *Contents of total phenols, tannins, flavonoids, and proanthocyanidins*

Phenolic compounds such as flavonoids, phenolic acids, and tannins are widely distributed in plants [47], which have gained much attention, due to their antioxidant activities and free radical scavenging activity or metal chelating ability [48], which potentially have beneficial implications for human health [49]. Table 3 summarizes the results from the quantitative determination of total phenols, tannins, flavonoids, and proanthocyanidins from myrtle seed extract and oil. Myrtle seed oil is characterized by the absence of flavonoids and proanthocyanidins; however, it contained small amounts of total phenols (0.25 mg GAE/g DW) and tannins (0.20 mg GAE/g DW). Methanolic extract had a high phenolic content (25.25 mg GAE/g DW) with the predominance of tannin fraction (20.33 mg GAE/g DW). However, flavonoids and proanthocyanidins were weakly present in methanolic seed extract with an amount of 0.75 mg CE/g DW for each sample. From the obtained results, it can be deduced that myrtle seed extract was particularly rich in hydrolyzable tannins because the proanthocyanidins (condensed tannins) were weakly present. Similar results were also reported by Aidi Wannes and Marzouk [22] for methanolic seed extract from *M. communis* var. *italica*.

3.4. *Antioxidant activity*

Free radical-scavenging properties of the methanolic extract and the oil are presented in Table 4. Lower IC_{50} value indicated

Table 2 – Fatty acid composition of different lipid classes of *Myrtus communis* var. *baetica* seed.

Fatty acid	Relative composition (% dry weight mean)			
	Total lipids	Neutral lipids	Glycolipids	Phospholipids
C12:0	0.24 ± 0.05 [c]	0.29 ± 0.05 [c]	1.89 ± 0.39 [a]	1.00 ± 0.32 [b]
C14:0	0.12 ± 0.01 [c]	0.26 ± 0.07 [b]	2.04 ± 0.44 [a]	1.85 ± 0.14 [a]
C16:0	10.17 ± 0.15 [b]	8.26 ± 0.41 [c]	16.44 ± 0.06 [a]	16.42 ± 0.08 [a]
C16:1 (*n*–7)	0.10 ± 0.01 [b]	0.14 ± 0.64 [b]	0.24 ± 0.13 [a]	0.07 ± 0.26 [c]
C18:0	3.13 ± 0.11 [b]	0.65 ± 0.60 [c]	4.76 ± 0.10 [a]	4.65 ± 0.40 [a]
C18:1 (*n*–9)	7.73 ± 0.12 [c]	4.31 ± 0.86 [d]	11.24 ± 0.05 [a]	9.83 ± 0.50 [b]
C18:2 (*n*–6)	77.88 ± 0.33 [b]	84.67 ± 0.23 [a]	58.12 ± 0.34 [d]	62.13 ± 0.26 [c]
C18:3 (*n*–3)	0.34 ± 0.01 [c]	0.32 ± 0.26 [c]	4.57 ± 0.24 [a]	3.26 ± 0.46 [b]
C20:0	0.14 ± 0.01 [c]	0.70 ± 0.07 [a]	0.24 ± 0.25 [b]	0.70 ± 0.14 [a]
C20:1 (*n*–9)	0.14 ± 0.01 [c]	0.36 ± 0.07 [b]	0.42 ± 0.33 [a]	0.04 ± 0.25 [d]
SFA	13.81 ± 0.01 [c]	10.16 ± 0.23 [d]	25.37 ± 0.24 [a]	24.80 ± 0.21 [b]
MUFA	7.97 ± 0.11 [c]	4.81 ± 0.52 [d]	11.90 ± 0.48 [a]	9.94 ± 0.42 [b]
PUFA	78.22 ± 0.25 [b]	84.99 ± 0.24 [a]	62.69 ± 0.27 [d]	65.39 ± 0.35 [c]
SFA/PUFA	0.18 ± 0.01 [a]	0.12 ± 0.27 [b]	0.40 ± 0.12 [c]	0.37 ± 0.27 [c]

[a–d] Values (mean ± standard deviation, $n = 3$) in the same column with different superscripted letters are significantly different at $p < 0.05$ (Duncan test).
MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid.

Table 3 – Total phenolic, tannin, flavonoid and proanthocyanidin contents of the methanolic extract and the oil from *Myrtus communis* var. *baetica* seed.

Samples	Relative content (mg/g dry weight)			
	Total phenols	Tannins	Flavonoids	Proanthocyanidins
Methanolic extract	25.25 ± 0.08 [a]	20.33 ± 0.05 [b]	0.75 ± 0.01 [c]	0.75 ± 0.01 [c]
Oil	0.25 ± 0.11 [a]	0.20 ± 0.12 [a]	—	—

Total phenolic and tannin contents were expressed by milligram gallic acid equivalent/gram; total flavonoid and proanthocyanidin contents were expressed by milligram catechin equivalents/gram.
[a–c] Values (mean ± standard deviation, $n = 3$) in the same column with different superscripted letters are significantly different at $p < 0.05$ (Duncan test).

higher antioxidant activity. The myrtle seed oil showed a lower antioxidant activity in the DPPH test, which could be due to a minor phenolic content. According to the findings of Ramadan et al [50], the antioxidant activity of seed oil is not only related to total phenolics but is also positively correlated with the total polar lipids that depend much on their functional groups, structures, and fatty acid composition. They also showed that phenols constitute the part of the polar fraction of seed oils and contribute to their antioxidant potential according to their concentration. Contrary to the oil, the methanolic extract of seed (IC_{50} = 0.01 mg/mL) showed higher scavenging ability on DPPH radicals when compared with that of the synthetic antioxidant BHT (IC_{50} = 0.02 mg/mL), suggesting the presence of specific bioactive components in myrtle seed that may be responsible for the antioxidant activity. Thus, it has been reported that the antioxidant activity is not only based on the total phenolics but also on the nature of the phenolic compounds [51]; besides, high-performance liquid chromatography analysis of the polyphenol of different fruit parts from *M. communis* var. *italica* showed that myrtle seed was rich in hydrolyzable tannins [22]. In a comparison of these results with those of our earlier study [52] concerning the antioxidant activity of myrtle leaf, flower, and stem extracts, we noted that seed, flower, and leaf extracts showed stronger scavenging ability on DPPH and that they were rich in hydrolyzable tannins. According to Yoshimura et al [53], hydrolyzable tannins exhibited the puissance antiradical activity in comparison with phenolic acids and flavonoids.

The degradation rate of β-carotene–linoleate depends on the antioxidant activity of the extracts. The sample with the lowest β-carotene degradation rate exhibited the highest antioxidant activity (Table 4). As for antiradical scavenging, seed extract showed a higher ability to prevent the bleaching of β-carotene than oil (IC_{50} = 40 mg/mL). BHT presented an IC_{50} (0.07 mg/mL) similar to that of seed extract, which means that use of synthetic antioxidants such as BHA or BHT could be avoided by replacing them with the natural ones. Moreover, the IC_{50} value found in myrtle seed extract was similar to that of leaf and flower extracts from Tunisian myrtle [52].

The reducing power of a bioactive compound may also serve as a significant indicator of its potential antioxidant activity [54]. As shown in Table 4, seed extract had higher reducing power (EC_{50} = 0.01 mg/mL) than ascorbic acid (EC_{50} = 0.04 mg/mL). The EC_{50} value found in myrtle seed extract was similar to that of leaf extract from Tunisian myrtle [52], indicating the presence of specific bioactive compounds, which were electron donors reacting with free radicals to convert them into more stable products and to terminate radical chain reactions as described by Shimada et al [55]. As for the antiradical scavenging and β-carotene degradation assays, the oil fraction was found to be a more powerful reducing agent than the corresponding methanolic extract fraction, which could be due to a smaller amount of phenols in oil. Similar results were found by Ishtiaque et al [56] who studied the antioxidant potential of the extracts, fractions, and oils derived from oilseeds.

Table 4 – Antioxidant activities of the methanolic extract and oil from *Myrtus communis* var. *baetica* seed.

	DPPH (IC_{50}, mg/mL)	β-Carotene bleaching (IC_{50}, mg/mL)	Chelating ability (IC_{50}, mg/mL)	Reducing power (EC_{50}, mg/mL)
Methanolic extract	0.01 ± 0.13 [a]	0.07 ± 0.25 [b]	3 ± 0.33 [b]	0.01 ± 0.15 [a]
Oil	10 ± 5.30 [c]	40 ± 1.14 [c]	—	20 ± 1.45 [c]
BHT	0.02 ± 0.20 [b]	0.07 ± 0.57 [b]		—
EDTA	—	—	0.03 ± 0.12 [a]	—
Ascorbic acid	—	—	—	0.04 ± 0.13 [b]
BHA	—	0.04 ± 0.15 [a]	—	—

EC_{50} value is the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was scavenged by 50%; and ferrous ions were chelated by 50%. The EC_{50} value was obtained by interpolation from linear regression analysis. BHT, EDTA, ascorbic acid and BHA were used as positive controls.
[a–c] Values (mean ± standard deviation, $n = 3$) in the same column with different superscripted letters are significantly different at $p < 0.05$ (Duncan test).
BHT = butylated hydroxytoluene; BHA = butylated hydroxyanisole; EC_{50} = half-maximal effective concentration.

The chelating ability (Table 4) was absent in the seed oil, whereas the methanolic seed extract presented a very low ability to chelate ferrous irons ($IC_{50} = 3$ mg/mL) in comparison to the positive control EDTA ($IC_{50} = 0.03$ mg/mL). According to Ebrahimzadeh et al [57], there was no correlation between the phenol content of an extract and its chelating activity. Therefore, they studied the iron chelating activity of some medicinal plants and found that some plant extracts with high content of phenol showed very little chelating ability, whereas the other ones with low phenol content showed good chelating ability.

3.5. *Conclusion*

This investigation provided a new insight into the chemistry of myrtle seed var. *baetica*, which was an important source of lipids, phenolics, and antioxidants for food industrial and biomedical applications.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

[1] Pottier-Alapetite G. Flore de la Tunisie. Angiospermes, dicotyledones dialypetales. Imprimerie officielle de la republique. Tunis, Tunisia: Scientifiques Tunisienne; 1979.

[2] Nuvoli F, Spanu D. Analisi e prospettive economiche dell'utilizzazione industriale del mirto. Riv ital EPPOS 1996;12:231–6.

[3] Mulas M, Spano D, Biscaro S, Parpinello L. Parametri di qualità dei frutti di mirto (*Myrtus communis* L.) destinati all'industria dei liquori. Ind Bevande 2000;29:494–8.

[4] Aidi Wannes W, Mhamdi B, Sriti J, Marzouk B. Glycerolipid and fatty acid distribution in pericarp, seed and whole fruit oils of *Myrtus communis* var. *italica*. Ind Crops Prod 2010;31:77–83.

[5] Mazza G. Gas chromatographic-mass spectrometric investigation of the volatile components of myrtle berries (*Myrtus communis* L.). J Chromatogr A 1983;264:304–11.

[6] Gauthier R, Gourai M, Bellakhdar J. A propos de l'huile essentielle de *Myrtus communis* L. var. *italica* recolte au Maroc. I. Rendements et compositions durant un cycle vegetatif annuel. Al Biruniya Rev Mar Pharm 1988;4:97–116.

[7] Jerkovic I, Radonic A, Borcic I. Comparative study of leaf, fruit and flower essential oils from Croatian *Myrtus communis* L. during a one-year vegetative cycle. J Essent Oil Res 2002;14:266–70.

[8] Flamini G, Cioni PL, Morelli I, Maccioni S, Baldini R. Phytochemical thypologies in some populations of *Myrtus communis* L. on caprione promontory (East Ligura, Italy). Food Chem 2004;85:599–604.

[9] Tuberoso CIG, Barra A, Angioni A, Sarritzu E, Pirisi FM. Chemical composition of volatiles in Sardinian myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils. J Agri Food Chem 2006;54:1420–6.

[10] Aidi Wannes W, Mhamdi B, Marzouk B. Essential oil composition of two *Myrtus communis* L. varieties grown in North Tunisia. Ital J Biochem 2007;56:180–6.

[11] Aidi Wannes W, Mhamdi B, Marzouk B. Variations in essential oil and fatty acid composition during *Myrtus communis* var. *italica* fruit maturation. Food Chem 2009;112:621–6.

[12] Pereira PC, Cebola MJ, Bernardo-Gil MG. Evolution of the yields and composition of essential oil from Portuguese myrtle (*Myrtus communis* L.) through the vegetative cycle. Molecules 2009;14:3094–105.

[13] Brada M, Tabti N, Boutoumi H, Wathelet JP, Lognay G. Composition of the essential oil of leaves and berries of Algerian myrtle (*Myrtus communis* L.). J Essent Oil Res 2012;24:1–3.

[14] Aidi Wannes W, Marzouk B. Maturational effect on essential oil yield and composition of *Myrtus communis* var. *baetica* fruit. J Essent Oil Bearing Plants 2012;15:847–53.

[15] Kafkas E, Güney M, Sadighazadi S, Yıldırım H, Kefayati S. Volatile compounds of selected white and black myrtle (*Myrtus communis L.*) types from Mediterranean region of Turkey. J Med Plants Res 2013;7:1244–8.

[16] Aidi Wannes W, Mhamdi B, Sriti J, Bettaieb I, Saidani Tounsi M, Marzouk B. Fatty acid and glycerolipid changes during Tunisian myrtle (*Myrtus communis* var. *italica*) fruit ripening. J Food Biochem 2011;35:177–94.

[17] Vacca V, Piga A, Del Caro A, Fenu PA, Agabbio M. Changes in phenolic compounds, colour and antioxidant activity in industrial red myrtle liqueurs during storage. Nahrung 2003;47:442–7.

[18] Alamanni MC, Cossu M. Radical scavenging activity and antioxidant activity of liquors of myrtle (*Myrtus communis* L.) berries and leaves. Ital J Food Sci 2004;16:197–208.

[19] Montoro P, Tuberoso CI, Perrone A, Piacente S, Cabras P, Pizza C. Characterisation by liquid chromatography-electrospray tandem mass spectrometry of anthocyanins in extracts of *Myrtus communis* L. berries used for the preparation of myrtle liqueur. J Chromatogr A 2006;1112:232–40.

[20] Amensour M, Sendra E, Abrini J, Pérez-Alvarez JA, Fernández-López J. Antioxidant activity and total phenolic compounds of myrtle extracts. [Actividad antioxidante y contenido de compuestos fenólicos totales en extractos de myrtus]. CyTA - J Food 2010;8:95–101.

[21] Tuberoso CIG, Rosa A, Bifulco E, Melis MP, Atzeri A, Pirizi FM, Dessi MA. Chemical composition and antioxidant activities of *Myrtus communis* L. berries extracts. Food Chem 2010;123:1242–51.

[22] Aidi Wannes W, Marzouk B. Differences between myrtle fruit parts (*Myrtus communis* var. *italica*) in phenolics and antioxidant contents. J Food Biochem 2013;37:585–94.

[23] Kanoun K, Belyagoubi Benhammou N, Ghembaza N, Atik Bekkara F. Comparative studies on antioxidant activities of extracts from the leaf, stem and berry of *Myrtus communis* L. Int Food Res J 2014;21:1957–62.

[24] Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res 2002;519:103–9.

[25] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 2006;97:654–60.

[26] Ma DWL, Wierzbicki AA, Field CJ, Clandinin MT. Preparation of conjugated linoleic acid from safflower oil. J Am Oil Chem Soc 1999;76:729–30.

[27] Belury MA. Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. J Nutr 2002;132:2995–8.

[28] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.

[29] Mangold HK. Thin layer chromatography of lipids. J Am Oil Chem Soc 12964;47:762–773.

[30] Lepage M. Identification and composition of turnip root lipids. Lipids 1967;2:244–50.
[31] Cecch G, Biasini S, Castano J. Methanolyse rapide des huiles en solvant. Note de laboratoire. Rev Fr Corps Gras 1985;4:163–4.
[32] Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 2002;50:3010–4.
[33] Hagerman AE, Butler LG. Protein precipitation method for the quantitative determination of tannins. J Agric Food Chem 1978;26:809–12.
[34] Sun B, Richardo-da-Silvia JM, Spranger I. Critical factors of vanillin assay for catechin and proanthocyanidins. J Agric Food Chem 1998;46:4267–74.
[35] Hanato T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull 1988;36:1090–7.
[36] Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem 2004;85:633–40.
[37] Koleva II , Teris AB, Jozef PH, Linssen AG, Lyuba NE. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal 2002;13:8–17.
[38] Yamaguchi F, Ariga T, Yoshimira Y, Nakazawa H. Antioxidant and antiglycation of carbinol from *Garcinia indica* fruit rind. J Agric Food Chem 2000;48:180–5.
[39] Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L, Lin Y, Fan W, Gu G. Effect of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in Barely (*Hordeum vulgare* L.). J Agric Food Chem 2006;54:7277–86.
[40] Zou YP, Lu YH, Wei DZ. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. J Agric Food Chem 2004;52:5032–9.
[41] Oyaizu M. Studies on products of the browning reaction prepared from glucose amine. Japanese J Nutr 1986;44:307–15.
[42] Statsoft. STATISTICA for Windows (computer program electronic manual). Tulsa, OK: StatSoft Inc; 1998.
[43] Ramadan MF, Morsel JT. Phospholipid composition of niger (*Guizotia abyssinica* Cass.) seed oil. J Food Sci Technol 2003;36:273–5.
[44] Shahinaz H, Hifza A, Bushra K, Khan JI. Lipid studies of *Cuminum cyminum* fixed oil. Pak J Bot 2004;36:395–401.
[45] Chahed T, Hamrouni I, Dhifi W, Msaada K, Kchouk ME, Marzouk B. Lipid evaluation during the development of pistachio seed from the region of Kairouan (Middle of Tunisia). J Food Lipids 2006;13:375–89.
[46] Finley JW, Shahidi F. The chemistry, processing and health benefits of highly unsaturated fatty acids: an overview. In: John WJ, Shahidi F, editors. Omega-3 fatty acids, chemistry, nutrition and health effects. Washington, DC: American Chemical Society; 2001. p. 1–13.
[47] Li BB, Smith B, Hossain MM. Extraction of phenolics from citrus peels: I. Solvent extraction method. Sep Purif Technol 2006;48:182–8.
[48] Lou SN, Hsu YS, Ho CT. Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. J Food Drug Anal 2014;22:290–5.
[49] Govindarajan R, Singh DP, Rawat AKS. High-performance liquid chromatographic method for the quantification of phenolics in 'Chyavanprash' a potent Ayurvedic drug. J Pharm Biomed Anal 2007;43:527–32.
[50] Ramadan MF, Krohl LW, Morsel JT. Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.), and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. J Agric Food Chem 2003;5:6961–9.
[51] Meda A, Lamiena CE, Romitob M, Millogoc J, Nacoulmaa OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 2005;91:571–7.
[52] Aidi Wannes W, Mhamdi B, Sriti J, Ben Jemia M, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. Food Chem Toxicol 2010;48:1362–70.
[53] Yoshimura M, Amakura Y, Tokuhara M, Yoshida T. Polyphenolic compounds isolated from the leaves of *Myrtus communis*. J Nat Med 2008;62:366–8.
[54] Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defensive system of various senescing leaves. J Agric Food Chem 1995;43:1813–5.
[55] Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945–8.
[56] Ishtiaque S, Khan N, Siddiki MA, Siddiki R, Naz S. Antioxidant potential of the extracts, fractions and oils derived from oilseeds. Antioxidants 2013;2:246–56.
[57] Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr J Biotechnol 2008;7:3188–92.