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Phytochemical analysis of *Myrtus communis* plant: Conventional versus microwave assisted-extraction procedures

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Abstract:
Background: Myrtle (*Myrtus communis L*) may constitute an interesting dietary source of health protective compounds. Microwave-assisted extraction (MAE) of total phenolic compounds (TPC) from myrtle leaf, stems, pericarp, and seeds was studied and the results were compared with those of the conventional method extraction (CME) in terms of extraction time.
Methods: Extraction yield/efficiency and antioxidant activity were measured using radical scavenging assay (DPPH$^{\bullet}$) and reducing power.
Results: The results show that the MAE was higher in terms of saving energy, extraction time (62 s) and extraction efficiency of bioactive compound compared to CME (2 h). Leaf presented the optimum content of total phenols (250 mg GAE.g^{-1} DW) and flavonoids (13.65 mg GAE.g^{-1} DW). However, the anthocyanin content was most important in pericarp extract (176.50±2.17 mg Cyd-3-glu g^{-1} DW). The antioxidant activity was important in all parts, mainly in leaves. The results indicated that appropriate microwave treatment could be an efficient process to phenolic compounds recovery and thus, better the antioxidant activity of myrtle extract.
Conclusions: Principal component analysis (PCA) applied to the experimental data shows that the distribution of the myrtle phenolic compounds depended on their plant part localization as well as the extraction method.

Keywords: antioxidant activity, microwave-assisted extraction, *Myrtus communis*, phenolic compounds
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Introduction

Myrtle (*Myrtus communis L*) belongs to the family of Myrtaceae and is an evergreen shrub, which grows wild in several regions all over the world [1]. Different parts of this plant found various uses in the food industry, such as for savoring meat and sauces, and in the cosmetic industry [2]. The leaf decoction was used for virginal washing enemas and against respiratory diseases [3]. A decoction or infusion of leaves and fruits of this plant were used as stomachic, hypoglycemic, cough, infectious, and oral diseases. They were also used for constipation, appetizing envy, and externally as anti-hemorrhagic for wound healing [1]. Regarding the chemical composition, previous studies on *Myrtus communis* aerial parts have revealed the presence of several compounds. Leaves and flowers contain essential oils, tannins, phenolic acids, and flavonoids [4, 5]. Fruits are mostly composed of volatiles oils, tannins, anthocyanins, fatty acids, sugars, and organic acids such as citric and malic acids [6].

Extraction represents the primary step to get a crude extract from plants; then the obtained extracts should undergo further analysis of their active components. Different techniques such as conventional method extraction (CME) methods including soaking, maceration [7, 8], water percolation, soxhlet extraction have been used [9]. These techniques are based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds. Moreover, these techniques require longer extraction time and as a result cause thermal degradation for most of constituents [10]. Over the past decade, various novel extraction

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techniques have been introduced and investigated, most of which were claimed to be better in terms of efficiency, extraction time, and solvent consumption. The techniques are microwave-assisted extractions (MAE) [8], supercritical fluid extraction (SFE), pressurized solvent extraction (PSE), and ultrasonic extraction (UE) [9].

MAE has drawn significant research attention in various field, in particular medicinal plant research, due to its special heating mechanism, moderate capital cost, and its good performance under atmospheric conditions. MAE is an innovative solvent extraction technology which offers a better alternative to several thermal applications due to its efficient volumetric heat production, and the fact that it has many advantages over CME, such methods improved efficiency, reduced extraction time, lower solvent consumption, higher selectivity toward target molecules, and higher level of automation [7]. In addition, a wider range of solvents can be used in MAE, as the technique which is less dependent on solvent affinity [11].

Several investigations have focused on the natural antioxidants compounds of Myrtle leaves [5, 12]. However, the emphasis has been given to the conventional extraction method. To our best knowledge, no literature report exists on the MAE of natural phenolic contents from different parts of myrtle (stem, pericarp, and seed) with exception of the study by Dahmoune, Nayak [13] in the case of myrtle leaf polyphenols. Since this is the most common form of using the species and it may constitute an interesting dietary source of health protective compounds. Therefore, the aim of the present work is (i) to compare the effects of MAE and CME on the extraction efficiency from different myrtle parts (in terms of TPC, flavonoids, anthocyanins, and condensed tannins) and (ii) to estimate the recovery and the antioxidant capacity of the extracts.

Materials and methods

Chemicals

Sodium carbonate (Na_2CO_3), Folin–Ciocalteau's phenol reagent and disodium hydrogen phosphate (Na_2HPO_4), aluminum chloride ($AlCl_3$) were obtained from Prolabo (Loire, France), and 1,-diphenyl-2-picrylhydrazil (DPPH) from Sigma Aldrich (Germany). Gallic acid, ferric chloride ($FeCl_3{\cdot}6H_2O$), potassium ferricyanide ($C_6N_6FeK_3$), trichloroacetic acid and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Biochem-chemopharma (Loire, France). All solvents used were of analytical grade.

Plant material and sample preparation

Myrtus communis L. samples were collected at optimal fruit maturity (November, 2012), from Addekar (Bejaia, North East of Algeria; 36° 41′ 32.54″ N, 4° 40′ 21.44″ E; altitude 1092 m). A voucher specimen has been stored in the Plant Ecology Herbarium of Es-Senia University (Oran, Algeria), under voucher number 1856. Leaves, stems, and fruits were isolated manually from the aerial parts. The samples were washed with a tap water then distilled water to remove any adhering soil and dust. Myrtle parts were dried at room temperature in a ventilated darkroom to protect the active compounds from light oxidation. The drying time was about 5, 3, and 7 days for the leaves, stems, and fruits, respectively. Fruit samples were peeled manually and seeds were recovered. All parts were ground with an electrical grinder (IKA modelA11 Basic, Staufen, Germany). The obtained powder was passed through standard 250 nm sieve and only the fraction with particle size < 250 nm was used. The powder was stored in airtight bags until use.

Extraction procedures of phenolic contents

Microwave-assisted extraction

Phenolic contents were extracted using a domestic microwave oven (Samsung MW813ST, Kuala Lumpur, Malaysia) modified with the addition of a condenser, generated during extraction procedure. It operates at a frequency of 2450 MHz and a maximum output power of 1000 W with a 100 W increment. The size of the heating cavity is 37.5 cm (L)×22.5 cm (W)×38.6 cm (D). For the extraction, a volume of 32 mL of ethanol/water (42/58, v/v) was added to 1 g of the powders in flat-bottomed flask. The mixture was irradiated at 500 W for 62 s (optimization conditions) then filtered with a sintered glass at 0.45 μm using a vacuum pump. The obtained extract was stored at 4 °C until further analysis [8].

Conventional method extraction

Regarding the CME, 1 g of each powder was placed in a conical flask, and 50 mL of ethanol/water (42/58, v/v) was added. After stirring for 2 h, the mixture was vacuum filtered. The obtained extract was stored at 4 °C until further analysis [8].

Phytochemical analysis

Total phenolic content

The total phenolic content (TPC) in the extracts was assessed according to the method of George, Brat [14]. Briefly, 500 µL of diluted and filtered extract from the different parts was added to 2.5 mL of 10-fold diluted (v/v) Folin–Ciocalteau reagent. The solution was mixed and incubated at room temperature for 2 min. 2 mL of 7.5 % (m/v) sodium carbonate was added and the solution was then incubated at 50 °C for 15 min. The absorbance of the sample was measured at 760 nm against a blank using a UV-VIS Spectrophotometer (SpectroScan 50, Nkesia, Cyprus). The assay was performed in triplicate. For quantification, a calibration curve was generated with the standard solution of gallic acid (R^2=0.998). The TPC was expressed as mg of gallic acid equivalent (GAE) per gram of dry weight (DW) basis (mg GAE g^{-1} DW).

Total flavonoid content

The total flavonoid content was estimated by the aluminum chloride method according to Quettier-Deleu, Gressier [15], based on the formation of a flavonoid–aluminum complex. Briefly, 1 mL of different extracts was mixed with 1 mL of 2 % (m/v) aluminum chloride. After 15 min of incubation in the dark, the absorbance of the mixture was measured at 430 nm. Each analysis was carried out in triplicate. The total flavonoid content was calculated from a calibration curve made with rutin (R^2=0.997) and expressed as milligrams of rutin equivalent per gram of dry weight (DW) basis (mg RE g^{-1} DW).

Total monomeric anthocyanin content

Total monomeric anthocyanin content was determined by the pH-differential method of Lee, Durst [16], based on the structural change of the anthocyanins chromospheres between pH 1.0 and 4.5. The absorbance was measured at 520 nm and at 700 nm in buffers at pH 1.0 and 4.5 respectively. The concentration of anthocyanins was obtained using equation (eq. 1). Results are expressed on a cyanidin-3-glucoside basis.

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/g DW)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (1)$$

where

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5}$$

MW (molecular weight): 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu); DF: dilution factor; l: path length in cm; ε: 26 900 molar extinction coefficient, in L × mol^{-1}× cm^{-1}, for cyd-3-glu; and 10^3: factor for conversion from g to mg.

Condensed tannin content

The condensed tannin content was determined by the HCl-Vanillin method as described by Aidi Wannes et al. [17]. One mL of extracts was mixed with 5 mL of reagent (HCl+Vanillin). The mixture was kept in the dark room for 20 min. The absorbance was determined at 500 nm versus a blank. All analyses were performed in triplicate. Total tannins were expressed as mg catechin equivalents per gram of dry weight basis (mg CE•g^{-1} DW) through a calibration curve made against catechin standard (R^2=0.996).

Antioxidant activity

The antioxidant properties of the active compounds are very important due to the deleterious role of free radicals in foods and biological systems [18]. The antioxidant activity of all parts of plant (leaves, steams, pericarp, and seeds of fruits) was evaluated by DPPH• radical scavenging assay and reducing power test. The higher percentage inhibition test rate is the greater the hydrogen donating ability, thus the higher antioxidant activities.

Radical scavenging activity assay

The free radical scavenging activity (RSA) of the extracts was determined using the DPPH• [19]. It is a highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a color-less hydrazine (DPPH-H). The free RSA of an extract can be expressed as the percentage of DPPH reduced by a given amount of extract. The RSA was measured, following the method of Dudonné, Vitrac [20]. DPPH• radicals have an absorption maximum at 515 nm [19], which disappears with reduction by an antioxidant compound. A DPPH• solution in absolute methanol (60 M) was prepared, and 3 mL of this solution was mixed with 1 mL of the different extracts. The samples were incubated for 20 min at 37 °C in the dark, then, the decrease in absorbance at 515 nm was measured. The α-tocopherol served as a positive control. All the tests were performed in triplicate, and the inhibition rate was calculated according to (eq. 2).

$$\%Scavenging = \frac{(A_{control} - A_{extract})}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of DPPH• at = 0 min; $A_{extract}$ is the absorbance of DPPH•in the presence of the sample at t=20 min.

Reducing power

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 causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by the measurement of the absorbance at 700 nm [21]. One mL of different extracts was mixed with 2.5 mL of a 0.2 M (m/v) sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % (m/v) potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10 % (m/v) trichloroacetic acid was added. Finally, 1 mL of the obtained solution was added to 5 mL of distilled water and 1 mL of 0.1 % (m/v) ferric chloride ($FeCl_3$), the intensity of the blue green color was measured at 700 nm. Tests were carried out in triplicate.

Statistical analysis

The analysis of variance (ANOVA) was performed using XLSTAT release10 (Addinsoft, Paris, France), Tukey's multiple range test (HSD) was used to compare between TPC content and antioxidant activity means as affected by microwave-assisted extraction (MAE) or conventional methods extraction (CME). Principal component analysis (PCA) was performed to detect structure in the relationships between variables, allowing its classification and the separation of each part. All parameters analyzed were used as variables in PCA.

PCA is a multivariate ordination technique used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables. In this study, PCA was applied to the phenolic compounds, flavonoid, anthocyanins, tannin content, and antioxidant activity of different myrtle parts for the two Tunisian cultivars and two factors were selected justifying 68.73 % of total variance

Results and discussion

Phytochemical analysis

Total phenolic content

As one of the most important antioxidant plant components, phenolic antioxidants have been widely investigated in many medicinal plants [22]. Their antioxidant activity is believed to be mainly due to their redox properties [23]. The phenolic content of the different parts of the studied plant was presented in Figure 1. The results showed a clear difference in the distribution of bioactive compounds in all parts, which confirm those reported in the literature [1]. The main significant differences were found in TPC contents among different parts. In fact, leaf extract presented the higher TPC (63.11±0.35 mg GAE g^{-1} DW) compared to that of pericarp and stem extracts. Seed samples presented the lowest TPC (56.32±11.81 mg GAE g^{-1} DW) than the other myrtle parts. These results were in agreement with the finding of Aidi Wannes and Marzouk [5] showing that Tunisian myrtle leaves extract possessed the highest TPC (33.67 mg GAE g^{-1} DW) as compared to that of the stem (11.11 mg GAE g^{-1} DW). In the other hand, Gardeli, Vassiliki [24] showed that Greece myrtle leaves possessed higher TPC (373 mg GAE g^{-1} DW) compared to that found in the present work. However, Aidi Wannes and Marzouk, who quantified TPC of fruits parts from *M. communis* var. L., revealed a content of 23.87 mg GAE g^{-1} DW for seeds and 2.76 mg GAE g^{-1} DW for pericarp fruits, which are low compared to those obtained in the present study. These differences could be due to the cultivar plant, environmental factors, collection period, geographical origin [25], and the methods used for extraction [26].

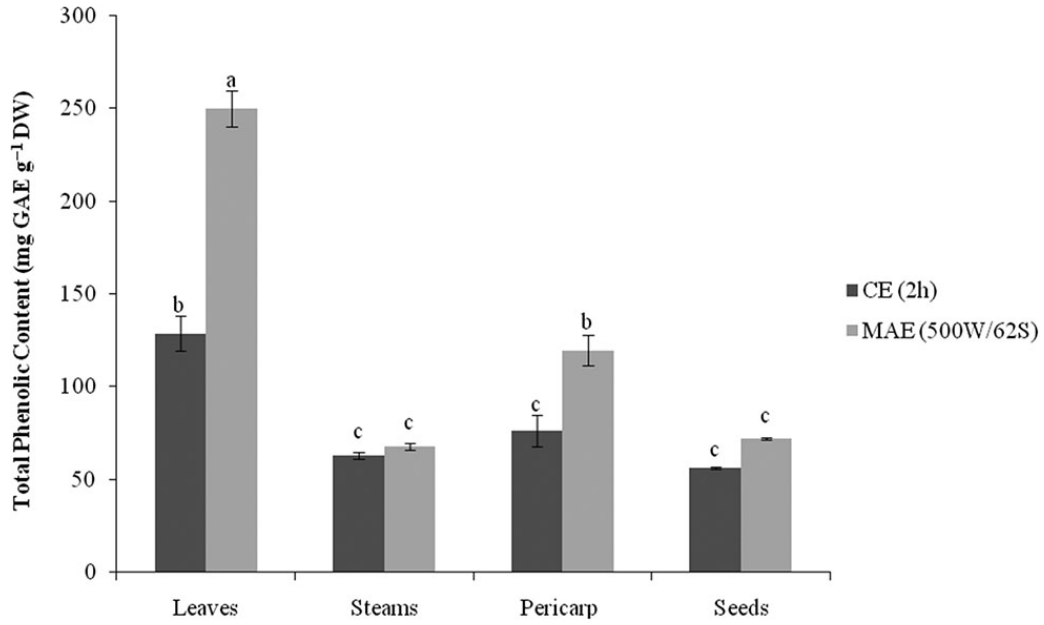

Figure 1: Total phenolic content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–c) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c.

To evaluate the MAE effects on the extraction efficiency, the amounts of phenolic contents obtained by MAE were comparable to those obtained by CME. The TPC of leaves and pericarp is significantly higher ($p<0.05$) than that obtained by CME. It increased from 128.73±6.84 to 249.86±9.2 mg GAE g^{-1} DW and from 76.38±7.27 to 119.60±8.4 mg GAE g^{-1} DW, respectively. However, stems and seeds have denoted no significant difference in their concentration (67.89±1.73 mg GAE g^{-1} DW, 72.06±0.81 mg GAE g^{-1} DW, respectively).

The high TPC obtained by MAE may be explained by the exposure of plant cells to microwave field. The dried plant material used for extraction contains traces of moisture and as microwave energy is absorbed and subsequently converted into heat, the moisture begins to evaporate. The vaporization of water generates pressure within the cell wall that eventually leads to cell rupture, thereby facilitating the leaching out of active constituents into the surrounding solvent and improving extraction yield [27]. To verify this fact, scanning electron microscopy (SEM) was employed by several authors to study the mechanism of MAE [28]. Dahmoune, Nayak [13] had treated myrtle leaves by scanning electron microscopy (SEM) after MAE, they showed that microwave heating caused a higher cellular damage helping the rapid release of solutes into the solvents and enhancing

the well-known main heating effect of microwaves. These results confirm that microwave radiations have a destructive effect on extraction sample matrix and the rapid extraction occurred when the active compounds elute and dissolve in solvent once the cell is ruptured.

Total flavonoids content

As can be seen in the Figure 2, flavonoid distribution within myrtle depends on the analyzed part. The highest values of flavonoid contents were observed in leaves and pericarp extracts, followed by seeds. However, the lowest flavonoid content was found in stem extract (0.74±0.01 RE g^{-1} DW). This finding is in contrast with the results of Aidi Wannes and Marzouk [5] who reported also that the highest values of flavonoids are in fruit pericarp. Compared CME with MAE, the yield increase in all parts namely in leaves (from 9.14±0.05 to 13.65±0.09 mg RE g^{-1} DW) and pericarp (6.95±0.20 to 11.50±0.26 mg RE g^{-1} DW).

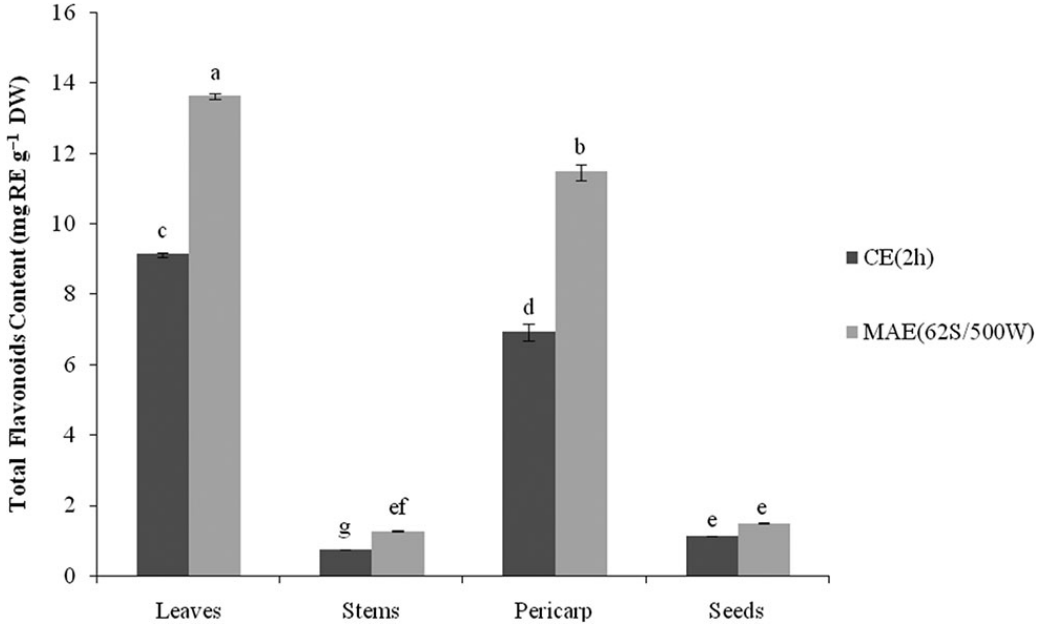

Figure 2: Total flavonoids content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–g) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c > d > e > f > g.

Total anthocyanin content

The distribution disparity of anthocyanin content was also found among different parts. Anthocyanins are the most important phytochemicals in myrtle plant and play an important role in its organoleptic properties [29]. Anthocyanins were predominant in pericarp extract (135.26±3.66 mg Cyd-3-glu g^{-1} DW) than in seeds (9.79±1.99 mg Cyd-3-glu g^{-1} DW). The lowest contents were observed in stem and leave extracts (1.00±0.13 mg Cyd-3-glu g^{-1} DW and 1.32±0.16 mg Cyd-3-glu g^{-1} DW, respectively) (Figure 3). These results are in agreement with those reported by [30]. The high anthocyanins content in myrtle pericarp and seeds could be explained by their increase during repining that is related to the change in the color of fruits surface from yellow-white to dark-blue. The anthocyanins concentration of microwave extracts is significantly different ($p<0.05$), they were higher than that obtained by CME, mainly in the pericarp extracts (176.50±2.17 mg Cyd-3-glu g^{-1} DW) than in the seeds (0.31±0.10 mg Cyd-3-glu g^{-1} DW). The farmer results agreed with those found by Jia et al. 2010, who report that microwave extraction increases the yield of anthocyanins compounds.

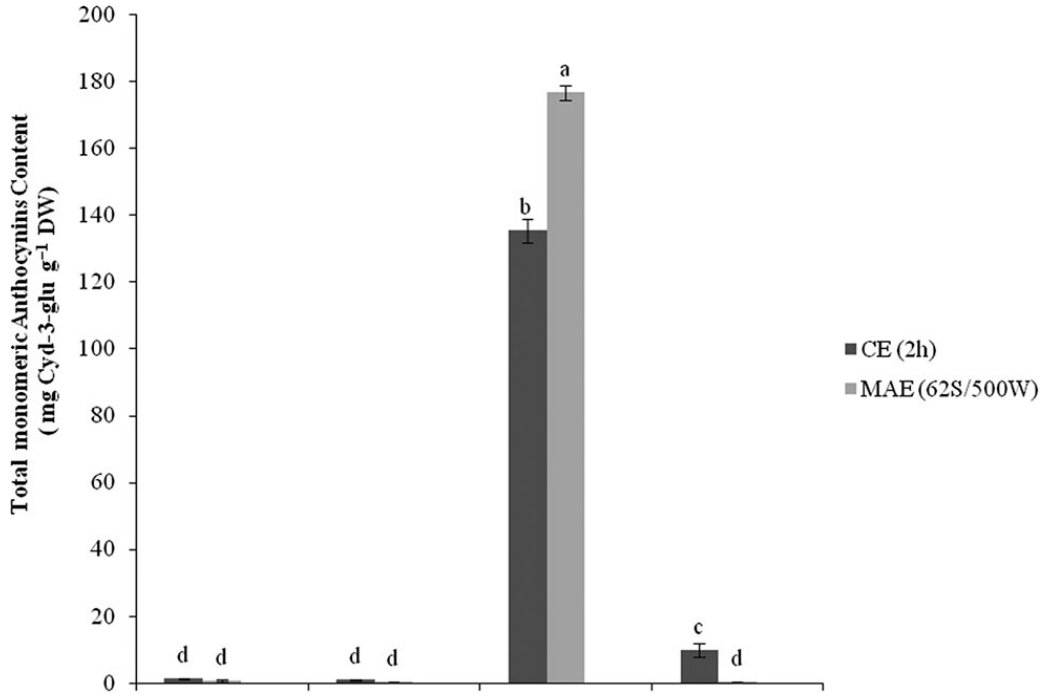

Figure 3: Total monomeric anthocyanin's content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–d) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c > d.

Total tannin content

The same discrepancy was also observed for condensed tannin content. In fact, tannin contents were higher in fruit pericarp, it is about 220.81±1.21 mg CE g^{-1} DW, while other parts presented a lower value, they were about 22.14±0.26; 35.74±0.26 and 36.01±0.20 mg CE g^{-1} DW for seeds, stem, and leaves extracts, respectively (Figure 4). Fruits are very astringent and are used as a condiment, a substitute for pepper, and considered as a rich source of tannins [31]. This result was in agreement with the work of Aidi Wannes and Marzouk [5] who studied the methanolic extract of the fruit from Tunisian myrtle, they reported that condensed tannin content was relatively low in pericarp and seeds, while the highest concentration was found in the whole fruit extract (0.96 mg CE g^{-1} DW). Compared CME with MAE, higher tannins content was observed in the pericarp (333.77±1.85 mg CE g^{-1} of DW). These results were in agreement with those found by Dahmoune, Nayak [13] and Jia, Dong, Dong [29] who report that microwave extraction increases the yield of tannin compounds from myrtle leaves.

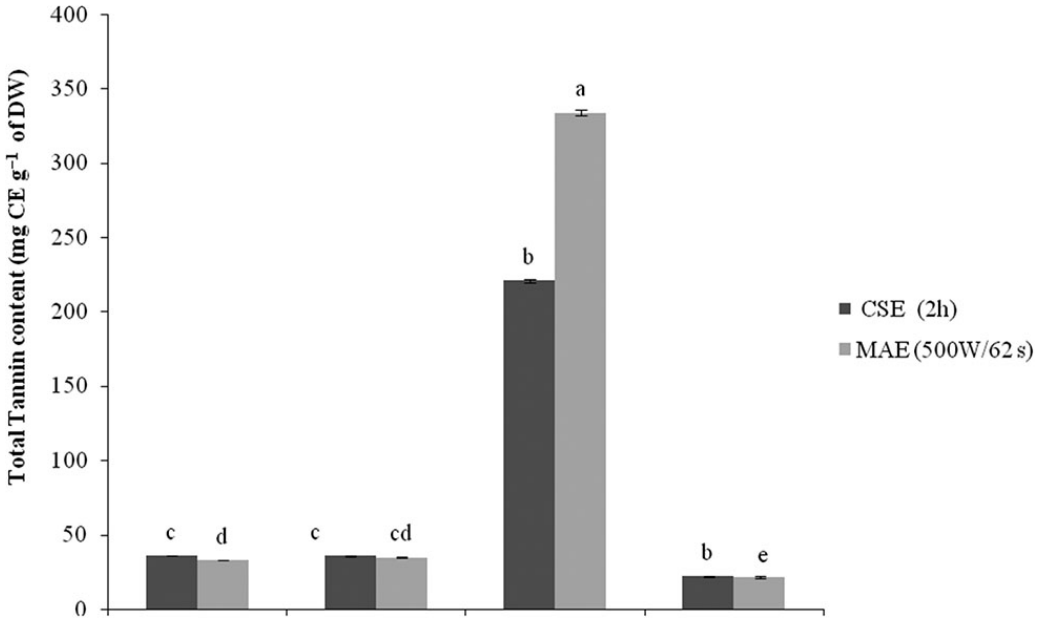

Figure 4: Total tannin content of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).
Values with different letters (a–e) were significantly different (Tukey, $p<0.05$) for the different aerial parts; the results are ranked in decreasing order: a > b > c > d > e.

The results of MAE at 500 W/62 s show that the amount of phenolic contents of leaves (63.15 %), stems (9.04 %), pericarp (43.73 %), and seeds of fruits (24.53 %) was higher than that obtained conventionally (1:50) for 7200 s. This results are in accordance with those reported in the literature [32]. Several authors reported the advantages of MAE compared to CME, such as reduced process time, lower solvent, energy demand, and higher yield [33, 34].

Conventional solvent extraction without microwave assistance is a time-consuming process that uses heat to increase the mass transfer rate of the extraction system [33]. The reduction of extraction time was due to the heating mechanism of microwave. It offers a rapid transfer of energy to the extraction solvent and raw plant materials [35]. A significant increase was obtained for the MAE as compared to that of the same sample extracted using the CME method. In addition, extraction time was significantly reduced in microwave extraction. Thus, microwave extraction method can be recommended for leaching phenolic compounds from myrtle.

Antioxidant activity

The amount of such compounds in each part of the plant is usually different. Myrtle was a source of natural antioxidants because of the activity of secondary metabolites, such as phenylpropanoids and essential oils. In the present study, the antioxidant activity of ethanolic extracts obtained by CME and MAE of myrtle leaf, stem, pericarp, and seeds of fruits was determined by measuring the free RSA (DPPH$^{\bullet}$) and the reducing power.

The effect of antioxidant on DPPH$^{\bullet}$ scavenging was conceived to their hydrogen donating ability [34]. The DPPH$^{\bullet}$ scavenging ability of the ethanolic extracts of myrtle parts was higher than that of α-tocopherol ($p<0.05$). The greatest antioxidant activity of the different parts of the studied plant was obtained in leaves extract (94.78±0.37 %) which is similar to that obtained by Ferchichi et al. [36] with a higher level in leaves of myrtle black fruit (86.54 %). The inhibition effect of DDPH$^{\bullet}$ radical by antioxidant from stems is about 88.72±0.65 %. The antioxidant activity of seeds was higher (88.41±0.64 %) than that of pericarp (88.03±0.37 %). Same results were reported by Aidi Wannes and Marzouk concerning the Tunisian myrtle fruits. Thus, it has been reported that free RSA is greatly influenced by the phenolic composition of the extract. The HPLC analysis of the phenolic contents of different fruit parts showed that the seed was rich in hydrolysable tannins, which were absolutely absent in the pericarp [5]. Additionally, Aidi Wannes and Marzouk [5] reported also that leaves were rich in hydrolyzable tannin. According to Yoshimura et al. [37], these hydrolyzable tannins exhibited a strong antiradical activity compared to other compounds such as gallic and quinic acids.

Concerning the reducing power activity, the presence of reductants (antioxidants) in the samples would result in the reduction of the Fe^{3+} ferricyanide complex to its ferrous form (Fe^{2+}) by donating an electron. Hence, the Fe^{2+} can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm [38]. Higher absorbance value indicates higher reducing power [39]. The results in Table 1 showed the Fe^{3+} reducing power

ability of ethanolic extracts from different parts. Leaves extracts had higher reducing power than pericarp ones. However, stems and seeds exhibited moderate reducing capacity.

Table 1: Antioxidant activity of different extracts of myrtle aerial parts extracted with microwave-assisted extraction (MAE) and conventional solvent extraction (CME).

Extraction Methods	Plant parts	DPPH•, %	Reducing Power, Abs
MAE	Leaf	88.35±0.47 [a]	0.661±0.002[b]
	Stem	87.09±0.28[b]	0.301±0.003[e]
	Pericarp	87.16±0.28[b]	0.439±0.006[d]
	Seeds	88.09±0.28[ab]	0.308±0.002[e]
CSE	Leaf	94.78±0.37[a]	0.865±0.001[a]
	Stem	88.72±0.65[ab]	0.406±0.0001[d]
	Pericarp	88.03±0.37[ab]	0.426±0.001[c]
	Seeds	88.41±0.64[ab]	0.442±0.0003[d]

All the values are mean±SD; SD, standard deviation. [a–e]Column wise values with same superscripts of this type indicate no significant difference ($p<0.05$).

The results shown in Table 1 suggest that the content of phenolic compounds can play a major role in the antioxidant activity of all extracts. Phenolic contents are the antioxidants that contribute to the high antioxidant capacity observed in different parts of plants [40]. Effectively, leaves extracts showed a higher antioxidant activity than other extracts, which correlated with its highest content of phenolic compounds. The different values of antioxidant activity obtained with each extract parts can be ascribed to their different chemical compositions [41]. MAE extraction gives the higher values of DPPH• scavenging capacity in leaves followed by those of seeds, pericarp than stem. The same tendency was observed using reducing power test.

Despite the high levels of phenolic contents obtained by microwave extraction, no correlation was observed between antioxidant activity and total phenolic content. Similar results were reported in the literature [42]. Furthermore, the study of Chiang et al. [43] reported that high TPC was not always correlated with high antioxidant activity. The type of extraction can explain this discrepancy, because the microwave radiations have an effect on the structure of phenolic compounds [11, 44]. The chemical nature affects also the content of polyphenols [26]. In addition, Hayat et al. [45] have reported that microwave irradiations could induce free radicals formation within the liquid medium, thus causing oxidation and degradation of the active compounds and the high phenolic content causes the association of the latter thereby preventing the DPPH• radical to access these compounds to induce antiradical activity. However, there is a correlation between DPPH• radical scavenging activity assay and reducing power test (r=0.11).

PCA analysis

PCA is a multivariate ordination technique used to display patterns in multivariate data. It aims to graphically display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables. In this study, PCA was applied to the phenolic compound; flavonoid, anthocyanins, tannin contents, and antioxidant activity of different myrtle parts and two factors were selected justifying 75.19 % of total variance. PC1 explained 46.32 % of the total variance in the dataset while PC2 explained 28.87 %.

The sample score plot for PC1 vs. PC2 is shown in Figure 5. Samples were located in four different plots, indicating their composition, and repartition part. The position of each variable in this loading plot describes its relationship to the other variables. Figure 5 shows four distinctive groups. The first and second groups are comprised of pericarp and leaves myrtle respectively, which are ported positively by PC1. The third group shows the positive correlation between CME leaves and DPPH, and reducing power test. The last group seed pericarp are ported negatively by PC1. Using the plots in Figure 5, it is possible to restart all phenolic compounds in myrtle part and to selected the adequate method of extraction. Phenolic compounds of pericarp are represented mainly by tannins 62.8 %, which are better extracted by MAE. In the other hand, microwave extraction of the TPC and flavonoids content in leaves was selected 72.2 % and 80.4 %, respectively. Concerning antioxidant activity, the DPPH and reducing power test were correlating positively with phenolic compound leaves obtained by CME. The anthocyanin compound was represented mainly in seed of fruit with a higher yield obtained by CME (56.2 %) in seeds pericarp and stem. The results of this study revealed the importance of comparing and exploring the variance of phenolic compound from different myrtle parts and extraction method.

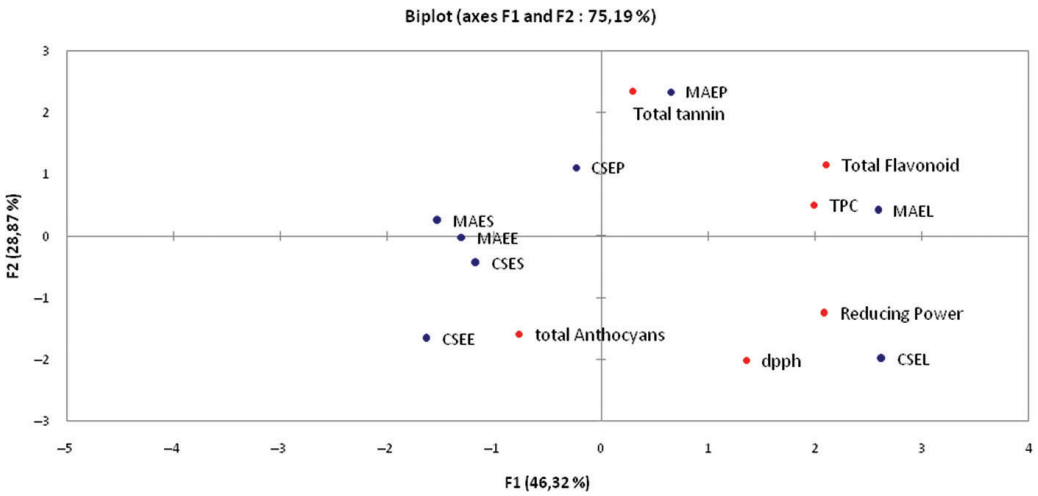

Figure 5: Principal component analysis of myrtle part samples based on the main important factors (MAE, CME, leaves, steams, pericarp, and seeds).

Conclusions

Myrtle aerial parts are potential source of active natural substances such as phenolic compounds. MAE was found to be highly effective enabling a considerable reduction in extraction time (62 s against 7,200 s), and the efficiency of extraction of phenolic contents from all myrtle parts was improved in comparison with the CME method with an increase of TPC but with antioxidant activity similar to those of CME. This showed great potential for industrial application in the near future.

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