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Total phenolic content and antioxidant activity of six wild *Mentha* species (Lamiaceae) from northeast of Algeria

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ABSTRACT

Objective: To investigate the total phenolics, flavonoids and tannins content and the *in vitro* antioxidant activities of methanolic extracts of six wild *Mentha* species which are *Mentha aquatica*, *Mentha arvensis*, *Mentha piperita*, *Mentha pulegium*, *Mentha rotundifolia* and *Mentha villosa*.

Methods: The Folin–Ciocalteu method was used to determine the total phenols content while flavonoids were estimated according to the aluminum chloride colorimetric method. To evaluate tannins content, vanillin and HCl were added to methanolic extracts. The antioxidant potential was measured by 1,1-diphenyl-2-picrylhydrazyl radical scavenging, ferrous ion chelating and the inhibition of β-carotene bleaching assays.

Results: The methanol extracts of Algerian mints were rich in phenolic compounds and exhibited powerful antioxidant activity ranging from 7.5 μg/mL to 44.66 μg/mL, which varied significantly among species. *Mentha aquatica* stood out with efficient antioxidant ability which was correlated to the high total phenolics content, followed by *Mentha arvensis* and *Mentha piperita* with very close values, comparing to *Mentha pulegium*, *Mentha rotundifolia* and *Mentha villosa* with lowest values.

Conclusions: These results show that methanolic extracts of *Mentha* species from Algeria have a great potential of polyphenols which can be used as a natural food preservative and antioxidant source.

1. Introduction

Most of aromatic and medicinal plants contain chemical compounds with antioxidant properties. Several studies that have been carried out on some of these plants have led to the development of natural antioxidant formulations for food, cosmetic, and other applications [1,2]. Among these natural compounds, phenols constitute one of the major groups of herbal compounds acting as radical scavengers and antioxidants [3,4]. Nowadays, scientific research reveals that the antioxidant properties of the plant extracts give beneficial effect to human health [5]. Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. Recently, interest has considerably increased in finding naturally occurring antioxidant to replace synthetic antioxidants, which were restricted due to their side effects such as carcinogenesis [6]. The Labiatae family includes about 220 genera and 3 300 species which are widely used for various purposes worldwide [7]. Plants belonging to the Labiatae family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties [8,9]. The genus *Mentha* is an important member of the family. It includes eighteen species and eleven hybrids,

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among which several species have economic importance due to their high-valued oil and good taste [10,11]. Mints are used in perfumery, confectionary and pharmaceutical preparations. They are also used in different traditional medicinal treatments as herbal remedies and in the food industry as food additives and taste enhancers because of their olfactory properties [12,13]. Infusion, decoction and hydrolat of the aerial parts of *Mentha* species have been used for centuries as tonics, carminative, digestive, stomachic, antispasmodic, and anti-inflammatory agents in Algerian folk medicine [14]. All these properties, typical of mints, have been referred to the combination of polyphenol derivatives [15]. Moreover, the *Mentha* species are cited as favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit reactive oxygen species attack on biological and food systems [16]. Based on these considerations, we aim to estimate the total phenolics, flavonoids, tannins content and the antioxidant activities of six wild *Mentha* species from Northeastern Algeria.

2. Materials and methods

2.1. Plant material

For each species, aerial parts of plants were harvested, at their flowering stage, from the National Park of El-Kala (Figure 1), which is one of the oldest and most important national parks of Algeria, situated in the extreme northeast of the country (El-Tarf region: 36°49′ N, 8°25′ E, rainfall: 910–1 200 mm per year). Seven of the ten sampling sites are Ramsar protected area and half of them are parts of the National Park of El-Kala as shown on Figure 1. Before analysis, samples were air-dried at room temperature (20–25 °C) for 2 weeks.

2.2. Methanolic extract preparation

For each species, 2 g of the air-dried aerial parts, finely ground in a mortar grinder mill, was extracted with 50 mL methanol (80% v/v) for 24 h in a water bath shaker maintained at room temperature. The extract was filtered using a 0.45 mm Millipore filter and stored in a brown bottle at 4 °C prior to further analysis [17].

2.3. Determination of total polyphenols content

The total polyphenol contents were determined using the Folin–Ciocalteu method [18] with modifications. Nearly 500 µL of dilute extract from each sample was mixed with 2 mL Folin–Ciocalteu reagent (diluted 10 times with distilled water). After 5 min, 2.5 mL of sodium carbonate solution (7.5%) was added and the mixture was allowed to stand for 90 min with intermittent shaking. The absorbance of the resulting solution was measured at 760 nm. The phenol contents were expressed in terms of milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

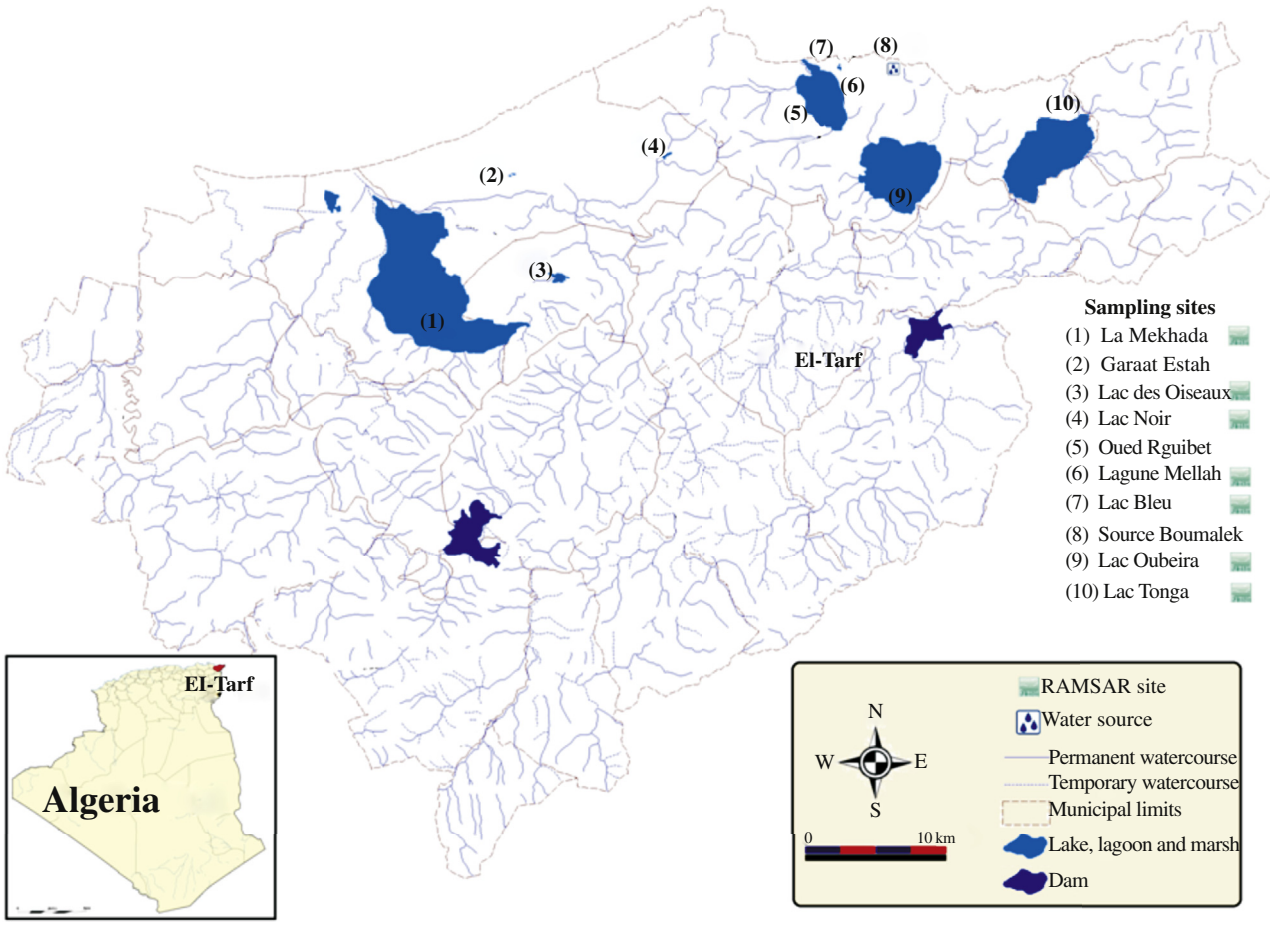

Figure 1. Sampling sites from El-Tarf region.

2.4. Determination of total flavonoids content

The total flavonoid contents were estimated according to the aluminum chloride colorimetric method [19]. Briefly, 500 μL of diluted extract was mixed with 500 μL of 2% $AlCl_3$ methanolic solution. After incubation at room temperature for 40 min, the absorbance was measured at 430 nm. Flavonoid contents were calculated from a calibration curve of rutin and expressed as milligrams of rutin equivalent per gram of dry weight (mg RE/g DW).

2.5. Estimation of condensed tannins

Total tannins were determined according to Rebaya *et al.* [20], with slight modifications. A volume of 12.5 μL of extract was added to 750 μL of vanillin and 375 μL of HCl. The mixture was then shaken and incubated at room temperature for 15 min. The absorbance was measured at 500 nm and the tannin content was expressed as milligrams of catechin equivalent per gram of dry weight (mg CE/g DW).

2.6. In vitro antioxidant activity

The *in vitro* antioxidant activity of the methanolic extracts was assessed with three different methods: the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, the ferrous ion chelating power and the β-carotene bleaching test.

2.6.1. DPPH radical scavenging assay

The DPPH radical scavenging capacity was measured [21]. In this study, 300 μL of each methanol extract was mixed with 900 μL of 0.4 mmol/L DPPH methanolic solution. The reaction was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm. The scavenging activity (SA) was estimated using the following equation:

$$SA\ (\%) = (A_{control} - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} is the absorbance of the tested sample. The concentration of extract that could scavenge 50% of the DPPH radicals (IC_{50}) was calculated. Trolox and butylhydroxytoluene (BHT) were used as positive references.

2.6.2. Ferrous ion chelating activity

The ferrous ion chelating activities of the methanolic extracts were measured according to Messaoud *et al.* [22]. In this study, 300 μL of different concentrations of each extract were added to 300 μL of $FeSO_4$ solution (0.1 mmol/L) and left for incubation at room temperature for 10 min. Then, the reaction was initiated by adding 300 μL of ferrozine (0.25 mmol/L). The mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was measured at 562 nm against a methanol blank. The ability of methanolic extracts to chelate ferrous ion was calculated using the following formula:

$$\text{Chelating effect}\ (\%) = (A_{control} - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the control sample (consisting of methanol, iron and ferrozine) and A_{sample} is the absorbance of the tested sample.

Results were expressed as IC_{50} (efficient concentration corresponding to 50% ferrous iron chelating). Ethylenediaminetetraacetic acid was used as a positive control.

2.6.3. Inhibition of β-carotene bleaching

A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 20 mL of chloroform. This solution (2 mL) was mixed with 20 mg linoleic acid and 200 mg Tween 40. After the chloroform was removed at 40 °C under vacuum, 50 mL of oxygenated ultra-pure water was added, and then the emulsion was vigorously shaken. Aliquots (750 μL) of this emulsion were transferred into different test tubes containing different concentrations of extracts (50 μL). As soon as the emulsion was added to each tube, zero time absorbance of the control, containing methanol instead of extract, was measured at 470 nm. The test samples were then incubated in a water bath at 50 °C for 120 min, when the absorbance was measured again. The β-carotene bleaching inhibition was calculated using the following equation:

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

where A_t and C_t are the absorbance values measured for the test sample and control, respectively, after incubation for 120 min, and C_0 is the absorbance value for the control measured at zero time during the incubation.

The results are expressed as IC_{50} values, the concentration required to cause a 50% β-carotene bleaching inhibition. BHT was used as a positive control [23].

2.7. Statistical analysis

All tests were performed in triplicate and results were expressed as mean ± SE. For each analysis, the results were compared by ANOVA followed by Duncan's multiple range test using SAS v. 9.1.3 program. The correlations between phenolic contents and antioxidant activities were assessed by the PROC CORR procedure using the same program. The chemical differentiation between species was evaluated by a principal component analysis (PCA) using the MVSP program. The significant difference was considered at $P < 0.05$.

3. Results

3.1. Total phenolic, flavonoid and tannin contents

All extracts had noticeable phenolic contents (Table 1). However, the total phenolic, flavonoid and tannin contents significantly varied among the studied species. The uppermost amounts were observed for *M. aquatica* (43.21 mg GAE/g DW, 31.77 mg RE/g DW and 8.67 mg CE/g DW) whereas minimum ones were noticed for *M. villosa* (14.66 mg GAE/g DW, 9.90 mg RE/g DW and 2.71 mg CE/g DW) for the total phenolic, flavonoid and tannin contents, respectively.

The total phenolic, flavonoid and tannin contents decreased in the following order: *M. aquatica* > *M. arvensis* > *M. piperita* > *M. pulegium* > *M. rotundifolia* > *M. villosa*.

Table 1
Total phenolics, flavonoids and tannins content of methanol extracts of the six *Mentha* species.

Content	*Mentha* sp.					
	M. aquatica	*M. arvensis*	*M. piperita*	*M. pulegium*	*M. rotundifolia*	*M. villosa*
Total phenolic contents (mg GAE/g DW)	43.21 ± 1.09[a]	32.90 ± 0.70[b]	31.40 ± 0.80[b]	17.00 ± 0.58[c]	15.10 ± 0.60[c]	14.66 ± 0.30[d]
Flavonoid contents (mg RE/g DW)	31.77 ± 0.19[a]	18.20 ± 0.20[b]	15.70 ± 0.10[c]	13.72 ± 1.00[d]	12.30 ± 0.30[d]	9.90 ± 0.32[e]
Tannin contents (mg CE/g DW)	8.67 ± 0.41[a]	7.33 ± 0.30[b]	6.50 ± 0.41[c]	5.20 ± 0.12[c]	3.05 ± 0.14[d]	2.71 ± 0.10[d]

In each line, values followed by different letters are significantly different ($P < 0.05$). *M. aquatica*: *Mentha aquatica*; *M. arvensis*: *Mentha arvensis*; *M. piperita*: *Mentha piperita*; *M. pulegium*: *Mentha pulegium*; *M. rotundifolia*: *Mentha rotundifolia*; *M. villosa*: *Mentha villosa*.

3.2. Antioxidant activity

The antioxidant activity of mint extracts was evaluated by measuring the free radical SA (DPPH), the ferrous ion chelating power and the β-carotene bleaching inhibition. All the extracts exhibited a noticeable effect which varied significantly among species (Table 2).

3.2.1. DPPH radical scavenging assay

The DPPH stable purple radicals react with suitable reducing agents (A–H), during which the electrons became paired off, yielding a stable diamagnetic molecule (yellow-colored diphenylpicrylhydrazine); the solution lost color, depending on the number of electrons taken up [24].

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants [25], thus a low value corresponds to a good scavenging ability. The results were expressed as IC_{50} value that is the amount of antioxidant necessary to decrease by 50% of the initial DPPH radical concentration.

The DPPH scavenging assay of the methanolic extracts obtained from the six Algerian *Mentha* species ranged from 7.50 μg/mL to 44.66 μg/mL (Table 2).

The results indicated that *M. aquatica* was the most efficient with 7.50 μg/mL followed by *M. arvensis* and *M. piperita*, whereas *M. pulegium*, *M. rotundifolia* and *M. villosa* were the least efficient. However, all extracts recorded low biological activities when compared to that reported for Trolox and BHT used as positive controls (Table 2).

3.2.2. Inhibition of β-carotene bleaching

Antioxidant activity determined by β-carotene/linoleic acid emulsified system capacity was estimated by inhibiting the formation of free radical in the β-carotene from oxidized linoleic acid, which was estimated spectrophotometrically by the decrease of the orange color characteristic of β-carotene, which is related to the inhibitory capacity of free radical formation.

The test of antioxidant activity by β-carotene/linoleic acid system also showed that *M. aquatica* stood out with IC_{50} 425.00 μg/mL, followed by *M. arvensis* and *M. piperita*. Hence, *M. pulegium*, *M. rotundifolia* and *M. villosa* were less effective (Table 2).

3.2.3. Ferrous ion chelating activity

The transition metal, iron, is capable of generating free radicals by Fenton reactions. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation was disrupted with the result that the red color of the complexes decreased. Metal chelating capacity was significant since the extract reduced the concentration of the catalyzing transition metal [26]. The chelating activity of samples on metal ions was determined by measuring the absorption of ferrozine-Fe^{2+} complex at 562 nm.

The results of the chelating iron indicated that *M. aquatica* had the largest ability to chelate iron (700.00 μg/mL), while *M. villosa* showed the lowest ability with 1 500.00 μg/mL (Table 2).

3.2.4. Correlation of antioxidant activities with phenolic contents

The correlation coefficients between phenolic contents and antioxidant activity of the six Algerian mints are shown in Table 3. The tannins content revealed the strongest correlation with the three antioxidant assays ($P < 0.05$), expressed by the highest coefficients in absolute values. The total polyphenolic

Table 3
Correlation of antioxidant activities with phenols, flavonoids and tannins content of methanol extracts of six *Mentha* species.

Antioxidant activities	Polyphenols	Flavonoids	Tannins
DPPH	$R = -0.908\,03$	$R = -0.823\,22$	$R = -0.963\,99$
	$P = 0.012\,3$	$P = 0.044\,1$	$P = 0.001\,9$
β-Carotene bleaching	$R = -0.852\,49$	$R = -0.752\,15$	$R = -0.952\,88$
	$P = 0.031\,0$	$P = 0.084\,5$	$P = 0.003\,3$
Chelating ion ability	$R = -0.877\,78$	$R = -0.781\,05$	$R = -0.973\,20$
	$P = 0.021\,5$	$P = 0.066\,7$	$P = 0.001\,1$

Table 2
Antioxidant activities of methanol extracts of the six *Mentha* species.

Antioxidant activities	*Mentha* sp.					
	M. aquatica	*M. arvensis*	*M. piperita*	*M. pulegium*	*M. rotundifolia*	*M. villosa*
DPPH [IC_{50} (μg/mL)]	7.50 ± 0.16[f]	13.33 ± 1.07[e]	17.00 ± 0.88[d]	25.66 ± 1.50[c]	31.66 ± 2.16[b]	44.66 ± 0.19[a]
β-Carotene bleaching [IC_{50} (μg/mL)]	425.00 ± 0.20[f]	466.00 ± 0.90[e]	516.00 ± 0.25[d]	599.00 ± 0.38[c]	763.00 ± 0.70[b]	939.00 ± 0.70[a]
Chelating ion ability [IC_{50} (μg/mL)]	700.00 ± 0.80[f]	800.00 ± 0.70[e]	873.00 ± 0.10[d]	1 000.00 ± 0.20[c]	1 300.00 ± 0.60[b]	1 500.00 ± 0.90[a]

Synthetic antioxidant: Trolox (5.8 ± 0.4); BHT (6.1 ± 0.8). Values are given as mean ± SD ($n = 3$). Values in each line followed by different letters are significantly different ($P < 0.05$).

contents were significantly and negatively correlated to the IC_{50} values ($P < 0.05$) consequently enhancing the antioxidant activity. Whereas flavonoid contents exhibited a significant correlation ($P < 0.05$) mainly with the DPPH test (Table 3).

3.3. Chemical differentiation among species

The chemical structure of the six *Mentha* species was evaluated through the PCA performed on the total polyphenol, flavonoid and tannin contents (Figure 2). The first two axes, explaining 99.75% of the variation, were defined, by total phenols (axis 1) and flavonoids (axis 2). The established plot segregated the studied species into 3 groups on the basis of their richness on phenolic compounds (Figure 2). The first group is defined by *M. pulegium*, *M. rotundifolia* and *M. villosa*. The second one comprised *M. arvensis* and *M. piperita*. *M. aquatica* constituted the third aggregate.

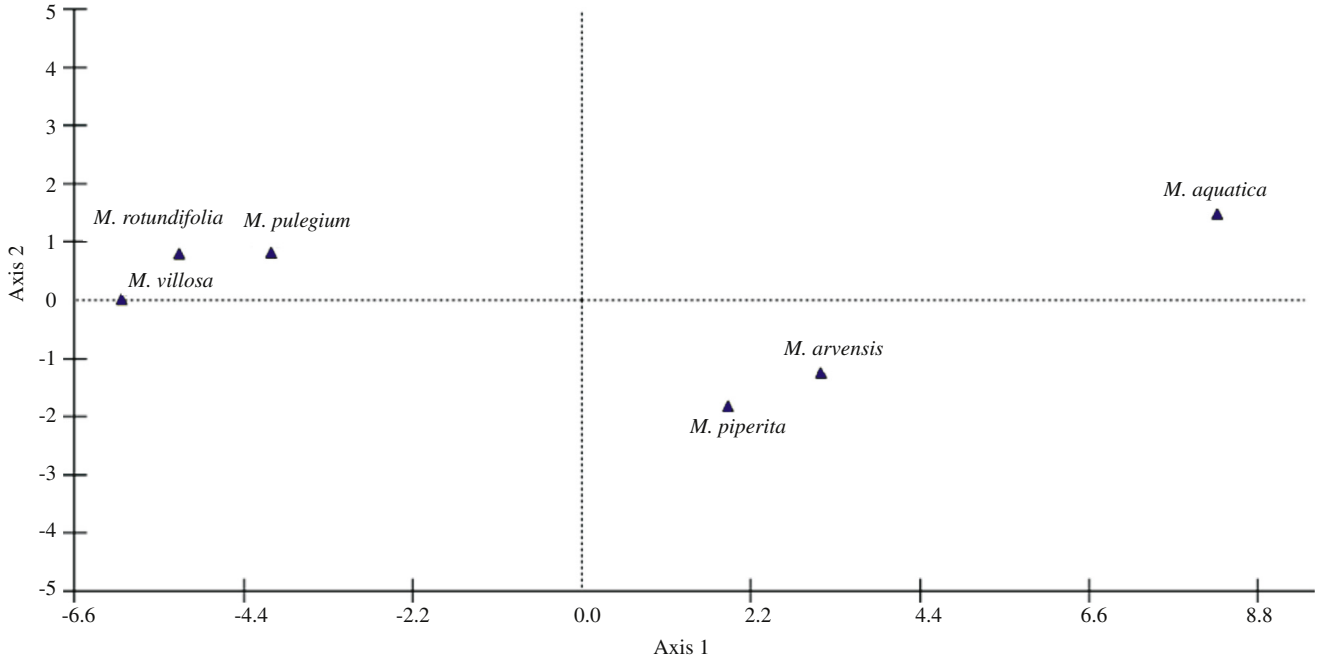

Figure 2. PCA of the six *Mentha* species.

4. Discussion

In the present study, we noticed that methanolic extracts of the six wild *Mentha* species from Algeria were rich in phenolics, flavonoids and tannins content thus the antioxidant ability of the six mints was important.

From our data, *M. aquatica* stands out with an efficient antioxidant ability which is correlated to the high total phenolic contents, followed by *M. arvensis* and *M. piperita* with very close content, comparing to *M. pulegium*, *M. rotundifolia* and *M. villosa* with lowest values. On the other hand, the correlation coefficients between phenolic contents and antioxidant activity of the six Algerian mints suggest that mint antioxidant activity is mostly correlated to tannins and polyphenols and less to flavonoids.

The high correlation between the values of phenols concentration in plant extracts and antioxidant activity is well documented [27,28]. In this regard, Luximun-Ramma *et al.* [29] showed a linear correlation between antioxidant activity and phenolic contents of the plant extracts.

Our results corroborate those of Nickavar [16] who reported that *M. piperita* had the highest total phenolic content followed by *M. pulegium* and *M. rotundifolia* for species grown in Iran. The same author reported that the sequence of activity is also indirect relationship with total phenol compounds, confirming that phenols are responsible for a significant part of their antioxidant and free radical scavenging effects. Similar results have also been observed by Dorman *et al.* [15] on aqueous extracts.

However, literature data on the phenolics content of *Mentha* species are frequently scattered throughout the papers and the available data are often difficult to compare because of methodological differences [30].

For instance, Ebrahimzadeh *et al.* [31], who used the percolation method with ethanol–water found that *M. aquatica* [(156.15 ± 6.40) mg GAE/g DW, (17.05 ± 0.63) quercetin equivalent (QE)/g DW] had higher total phenolic content than *M. piperita* [(13.4 ± 0.3) mg GAE/g DW, (122.3 ± 4.1) QE/g DW] but lower values for flavonoids were recorded.

Brahmi *et al.* [30] reported that ethanolic extracts of *M. pulegium* [(6.1 ± 0.5) mg GAE/g DW, (0.85 ± 0.01) QE/g DW] are richer than *M. rotundifolia* for the total phenolics but lower for flavonoids content [(3.3 ± 0.1) QE/g DW].

The antioxidant activity of polyphenols is mainly due to their ability to act as hydrogen donors, reducing agents and radical scavengers [32]. This activity is generally dependent on total phenol content [33]. In fact, the methanolic extract of *M. aquatica*, which had the highest phenolic, flavonoid and tannin contents, showed the greatest antioxidant activities. Furthermore, it should be taken into consideration that antioxidant capacities might be attributed to the chemical structure of compounds, as well as synergistic or antagonistic effect of compounds present in the crude extract [34]. Accordingly, the variation of antioxidant capacity between *Mentha* species, observed in our study, could be explained by their polyphenolic content.

The methanol extracts of *Mentha* species evaluated in this study showed considerable antioxidant levels correlated to the

strong polyphenols content. The present study furnished additional data sustaining the use of wild *Mentha* species as herbal tea or additive in foods and folk medicine for the therapy of infectious diseases.

A further study of the characterization of the phenolic profiles of these *Mentha* species with tests *in vivo* conditions is also required to strengthen antioxidant abilities which may be used for conservation of raw and processed foods as well as pharmaceuticals and natural therapies of infectious, human and plant diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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