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Antioxidant capacity and phenolic content of two Algerian *Mentha* species *M. rotundifolia* (L.) Huds, *M. pulegium* L., extracted with different solvents

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Abstract:
Background: It is important to consider the optimum conditions and processing factors (like solvent type) influencing activity of plant antioxidants for utilization in food and biological systems.
Methods: The antioxidant capacity and phenolic content of two *Mentha* species, namely, *Mentha pulegium* L. (MP) and *Mentha rotundifolia* (L.) Huds (MR), were studied and six solvent systems were used. The total antioxidant capacity of the mint species extracts was evaluated using phosphomolybdenum method and the free radical-scavenging capacity by 2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay.
Results: The efficiency of the used solvents to extract phenols from the two species varied considerably. The highest total phenolic content was obtained from methanol extract of MP (25.3±1.3 mg GAE/g_{dw}) and total flavonoid content from methanol extract of MR (10.1±0.1 mg QE/g_{dw}). High phenol content was significantly correlated with high antioxidant capacity. The methanol extracts showed the highest radical scavenging activity. All the extracts showed variable antioxidant capacity by the formation of phosphomolybdenum complex. Acetone extract of MP and methanol extract of MR exhibited marked reducing power in this method.
Conclusions: Our findings identified the appropriate solvent for extracting MP and MR phenolics which might provide a rich source of natural antioxidants.

Keywords: antioxidant activity, *Mentha pulegium*, *Mentha rotundifolia*, phenolic compounds, solvent extraction
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Introduction

There is an increasing interest in the measurement and use of plant antioxidants for scientific research as well as industrial (dietary, pharmaceutical, and cosmetic) purposes. This is mainly due to their strong biological activity, exceeding those of many synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which have possible activity as promoters of carcinogenesis [1].

It is in this connection that several studies have shown that foods of high antioxidant activity tend to improve health of the consumers by acting as free radical scavengers or reductors and such reduce the risk of chronic diseases [2]. Some antioxidant compounds are extracted from easy sources, like agricultural and horticultural crops or medicinal plants [1]. A wide spectrum of spices are grown and consumed in Algeria but not much information is available on their phenolics content of and their potential as sources of antioxidants.

Mentha pulegium L., and *Mentha rotundifolia* (L.) Huds are native to Africa, temperate Asia and Europe [3]. These two *Mentha* species grow spontaneously, are very abundant and very used in Algeria. *M. pulegium* L., called "Feliou" is widely used in practice as a digestive, cholegogic, carminative, pulmonary antiseptic, refreshing, tonic, appetizer, stomachic, choleretic, expectorant and bechic and aromatic and spasmolytic agent. Besides, the leaves and flowering tops are used against palpitations, intestinal fermentation, liver pain, dizziness, general weakness, hiccups, chronic bronchitis and obstinate cough [4].

M. rotundifolia, known as "Timija", is appreciated for its antiemetic, antidiarrhaea, antihemorrhoidal and its analgesic effects [5]. Moreover, it is widely used in Maghreb, primarily for external use [6].

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Mint species are a source of various biologically active compounds, such as phenolic compounds, which are responsible for most of the antioxidant activities of these plants [7]. However, the extraction method of phenolic compounds differs from plant to plant and an ideal extraction method for a particular phenolic source has to be individually designed and optimized [8].

Extraction efficiency is influenced by various factors such as a method of extraction, solvent type, contact time, extraction temperature, solid to solvent ratio and particle size [9]. Nevertheless, solvent type has a major importance in extraction efficiency. Solvent extraction is frequently used for isolation of antioxidant and extraction yield is dependent on the solvent and method of extraction, due to the different antioxidant potentials of compounds with different polarity [10].

To our knowledge, no comparative study on the effect of solvent in extracting phenolics from the two *Mentha* species studied has previously been conducted. On the other hand, most of the previous studies were restricted to the usage of single solvent for extraction, either methanol [11–15] or ethanol [7, 16–19]. Besides the above solvents, water has been used [7, 12].

Nevertheless, hydromethanolic or ethanolic solutions of 30–80 % (*v/v*) have been preferentially used for extracting phenolic acids or flavonoids in mints [20]. Besides, the mixture of methanol, acetone and ethanol with water (1:1) allowed the maximum extraction of phenolic compounds from other *Mentha* species [21, 22]. In this study, samples of two *Mentha* species (*M. pulegium* and *M. rotundifolia*) widespread in Algeria were used in order to retain their original medicinal quality for the accurate quantification. Each sample was extracted using six different polarities of solvent systems that are pure acetone, pure ethanol, pure methanol and these solvents with specific proportion of distilled water (1:1).

Therefore, the main objectives of this study were (i) to evaluate the efficiency of six different solvents (best solvent) in extracting of polyphenols and antioxidants from *Mentha* specimens and (ii) to determine possible correlation between antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) of the extracts.

Materials and methods

Plant materials

Plant materials were collected in August 2009 from Bejaia area located in Algeria. The Professor J. Lejoly, in the Laboratory of Systematical Botany and Phytosociology, Free University of Brussels (ULB) (Belgium), validated their prior identification based on their vernacular name. BR 0000006946043 and BR 000000 6946197 numbers for *M. pulegium* and *M. rotundifolia* respectively were accorded to the voucher specimen after deposition in the National Botanical Garden Herbarium of Meise (Belgium).

Chemicals

All chemicals and reagents used in this study are of analytical grade. Methanol, ethanol, acetone and Folin–Ciocalteu phenol reagents were purchased from VWR BDH Prolabo (Madrid, Spain), 1,1-diphenyl-2-picryhydrazyl radical (DPPH), gallic acid and quercetin were obtained from Sigma–Aldrich Chemicals (represented by Algerian Chemical Society, Setif, Algeria), anhydrous sodium carbonate (Na_2CO_3) and aluminum chloride ($AlCl_3$) were purchased from Biochem, Chemopharma (Montreal, Quebec).

Extraction of polyphenols

Phenolics extraction was performed as described by Brahmi Fatiha [23]. A required amount (5 g) of mint dry powder was soaked and shaken in 100 mL of solvent in conical flask (room temperature, 130 rpm, 24 h), using stirring plate (Velp Scientifica, Carnate, Italy). After filtration of the extracts through Whatman paper no. 1, the filtrate was evaporated under vacuum to dryness in a rotary evaporator (Buchi R 210, Switzerland) at 40 °C. Six different solvents were used for each sample extraction: acetone, ethanol, methanol, 50 % acetone (v/v), 50 % ethanol (v/v) and 50 % methanol (v/v).

The extraction yield was calculated as follows:

Yield (%) = $W_1 - W_0 / W_2 \times 100$

where W_0 was the weight of the empty conical flask, W_1 was the weight of the conical flask after dryness and W_2 was the weight of the dried powder of plant material.

Determination of TPC

TPC from the extracts were quantified using Folin–Ciocalteu's method [24]. Folin–Ciocalteu's reagent (0.5 mL) was added to 100 µL of extract. Then, 1.5 mL of 20 % (w/v) sodium carbonate was added to the mixture after 5 min of incubation at room temperature, followed by distilled water to a final volume of 10 mL. After 60 min of incubation at 25 °C, the absorbance was read at 760 nm against blank (solvent used for extraction). Gallic acid was used to plot the standard curve. The results were expressed as milligram gallic acid equivalent per gram dry weight of the sample (mg GAE/g_{dw}).

Determination of TFC

TFC was determined using the method described by Bahorun et al. [25]. An aliquot (1 mL) of extract was mixed with 1 mL of aluminum chloride (2 % w/v). After incubation at room temperature for 15 min, absorbance of the reaction mixture was measured against blank at 430 nm. Quercetin was used as a reference to produce a standard curve. The data were expressed as milligram quercetin equivalent per gram dry weight of the sample (mg QE/g_{dw}).

Phosphomolybdate assay (TAA)

The total antioxidant activity (TAA) of the extracts was evaluated by the phosphomolybdenum method according to Jayaprakasha and Patil [26]. Briefly, a 0.1 mL of sample aliquot was mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Test tubes were coated and incubated for 90 min in a water bath at 95 °C. After cooling at room temperature, the absorbance of the sample mixtures was measured at 695 nm against a blank. The antioxidant activity was expressed as the absorbance of the sample.

DPPH radical scavenging activity assay

Radical scavenging activity (RSA) of extracts was measured by the slightly modified method of Williams et al. [27], as described below. Briefly, a 0.1 mM solution of DPPH in ethanol was prepared. An aliquot of 100 µL of extract was added to 1 mL of the DPPH solution. For blank, only 100 µL of extraction solvent was added to the DPPH solution. After vigorous agitation, the solution was incubated for 30 min in the dark at room temperature. The absorbance was recorded at 517 nm, and then the scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

Scavenging effect % = [(control absorbance−sample absorbance)/(control absorbance)] × 100.

Statistical analysis

The experimental results were performed in triplicate. The data were recorded as mean±standard deviation. Statistical differences among the various phenolic compound extract contents and different antioxidant assays with least significance difference ($p<0.05$) were analyzed by one-way ANOVA test. Pearson correlation coefficient was used to obtain correlations. p values <0.05 were regarded as significant.

Ethics

All the research meets the ethical guidelines, including adherence to the legal requirements.

Results and discussion

Extraction yield, TPC and TFA

The highest yield was achieved by the polar solvents. The solvent polarities of used are listed in Table 1. A 50 % acetone extract of *M. pulegium* provided maximum yield of 14.2 %, whereas acetone provided minimum yield

of 2.9 %. This is in agreement with the reports of Alu'datt, Rababah [22] which showed that aqueous acetone (50 %) is effective solvent for extraction of phenolic compounds from *M. spicata*, while acetone provided the lowest phenolic compounds content. On the other hand, 50 % ethanol extract of *M. rotundifolia* gave maximum yield of 14.6 % and acetone gave minimum yield of 5.4 %. In studying *M. aquatica* phenolic content, Salmanian, Sadeghi Mahoonak [21] noted that in all extracts, the highest TPC was observed in 50 percentage concentration.

Table 1: Solvent effects on the yield of *Mentha* species extraction.

Extraction solvent	Snyder's solvent polarity index[a]	Extract yield, % g/g sample	
		M. pulegium	*M. rotundifolia*
Organic			
Methanol	6.6	12.9	10.0
Ethanol	5.2	4.3	6.0
Acetone	5.4	2.9	5.4
Aqueous			
50 % methanol (50:50 v/v methanol–water)	7.8	13.4	12.4
50 % ethanol (50:50 v/v ethanol–water)	7.1	13.7	14.6
50 % acetone (50: 50 v/v acetone–water	7.2	14.2	11.9

[a] Snyder's solvent polarity index cited from Ref. [31]. The aqueous solvent mixture indexes were calculated from equation $(I_A /100 \times P_A) + (I_B /100 \times P_B)$ where I_A and I_B are polarity index of solvents A and B, respectively, and P_A and P_B are percentage of solvents A and B, respectively, in the solvent mixture.

The difference in polarities of extracting solvents might influence the solubility of chemical constituents in a sample and its extraction yield [28]. Therefore, mixtures of alcohols and water have revealed to be more efficient in extracting phenolic constituents than compared to mono-component solvent system [29].

The difference between extraction yields obtained for the two species depended on the plant material analyzed. Variation in the yields of various extracts is attributed to polarities of different compounds present in the two different mints and the same differences have been reported in literature for spearmint [23].

The effects of the used solvent-systems in extracting polyphenolics and antioxidants from these plants were quantitatively measured and compared. Table 2 shows a list of the TPC. The means of TP in *M. pulegium* and *M. rotundifolia* leaf extracts in terms of mg GAE/g dw were ranged from 4.2 to 25.3 mg and from 3.1 to 20.8 mg, respectively. These results showed that, polyphenol content was strongly dependent on the solvents. Polar extracts of *M. rotundifolia* had more phenolics than non-polar ones. As mentioned above, our results showed clearly that a higher content of polyphenols was obtained with an increase in the polarity of the used solvent. Several studies have also revealed the same results in extracting phenolic from the dry-ground samples of plant materials [30, 31]. Whereas, methanol has been reported to be the most suitable solvent in the extraction of polyphenolic compounds from *M. pulegium*. Numerous researches have also showed the efficiency of methanol in extracting phenolic compounds from different plants [32–34]. According to Trabelsi, Megdiche [35] pure methanol is an effective solvent for antioxidant extraction, especially phenolic compounds, in contrast to pure ethanol that showed the lowest extraction power. In fact, extracting phenolics by aqueous mixtures of ethanol and acetone were respectively superior by 14- and 1.5-folds as compared to the same pure solvents, whereas methanol dilution induces a decrease (about −26 %) in the extraction capacity of leaf polyphenols. So, methanol has been reported to be the most suitable solvent in the extraction of polyphenolic compounds from plant tissue due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols [33].

Table 2: Total phenolic content (TPC) and total flavonoid content (TFC) in two *Mentha* species (*M. pulegium* (MP); *M. rotundifolia* (MR)) extracted using six different solvents.

Solvent	TPC				TFC			
	mg/g de MP	mg/g dw	mg/g de MR	mg/g dw	mg/g de MP	mg/g dw	mg/g de MR	mg/g dw
Methanol	126.3±6.4[a]	25.3±1.3[a]	63.7±1.2[e]	12.7±0.2[e]	25.5±3.1[e]	5.1±0. 6[d]	50.4±0.2[a]	10.1±0.1[a]
50 % methanol	78.5±5.2[d]	15.7±1.0[d]	100.5±8.6[c]	20.1±1.8[c]	9.5±1.3[c]	1.9±0. 2[h]	18.8±0.4[e]	3.8±0.1[e]
Ethanol	21.0±0.6[f]	4.2±0.1[f]	15.4±2.2[f]	3. 1±0.4[f]	10.5±0.8[f]	2. 1±0.2[g,h]	33.0±0.5[c]	6.6±0.1[c]

50 % ethanol	114.8±1.2^{b}	22.9±0.2^{b}	104.2±5.6^{c}	20. 8±1.1^{c}	11.5±2.0^{c}	2.3±0.4^{g,h}	20.8±1.9^{e}	4.2±0.4^{e}
Acetone	21.1±0.1^{f}	4. 2±0.03^{f}	16.0±1.6^{f}	3.2±0. 3^{f}	9.7±0.6^{f}	1. 9±0.1^{h}	40.0±1.6^{b}	8.0±0. 3^{b}
50 % acetone	116.4±6.5^{b}	23.3±1.3^{b}	100.6±2.4^{b}	20. 1±0.5^{c}	13.9±2.2^{c}	2.8±0.4^{f,g}	19.1±1.9^{e}	3.8±0.4^{e}

Values followed by different superscripts (a–h) in the rows are significantly different at p<0.05 (means of the replicates).

As for TPC, solvent extracting showed significant differences in TFC of the six solvent extractions from the two species. Most of the extracts contain a lower amount of TFC than of TPC. Flavonoids are considered as being restricted in distribution and composition in these plants in comparison with the overall phenolics. The TFC for MP and MR, expressed as mg QE/g dw, varied from 1.9 to 5.1 mg and from 3.8 to 10.1 mg, respectively.

Methanol allowed the extraction of a considerable amount of flavonoids (10.1±0.1; 5.6±0.6 mg QE/g dw for MR and MP respectively), however, which was almost twice as much as the amount extracted by ethanol (6.6±0.1; 2.1±0.2 for MR and MP respectively). This is in agreement with results reported by Casazza et al. [36]

For MR, the TFC of the methanol extract was followed by acetone and ethanol ones with significant difference at p<0.05 (acetone: 4.1±0.2; ethanol: 3.8±0.1). About MP except of methanolic extract, there was not a significant difference between all other extracts.

We noted that pure solvent extracts had the highest amount of flavonoids. This can be explicated by the nature of flavonoids present in the studied plants. The least polar solvents are considered to be suitable for the extraction of lipophilic compounds [16].

Antioxidant activity

The antioxidant activities of the extracts were evaluated using phosphomolybdate (TAA) and DPPH-free radical scavenging assays. In the phosphomolybdenum assay, which is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (TAA) [37], the extracts exhibited different degree of activity (Figure 1).

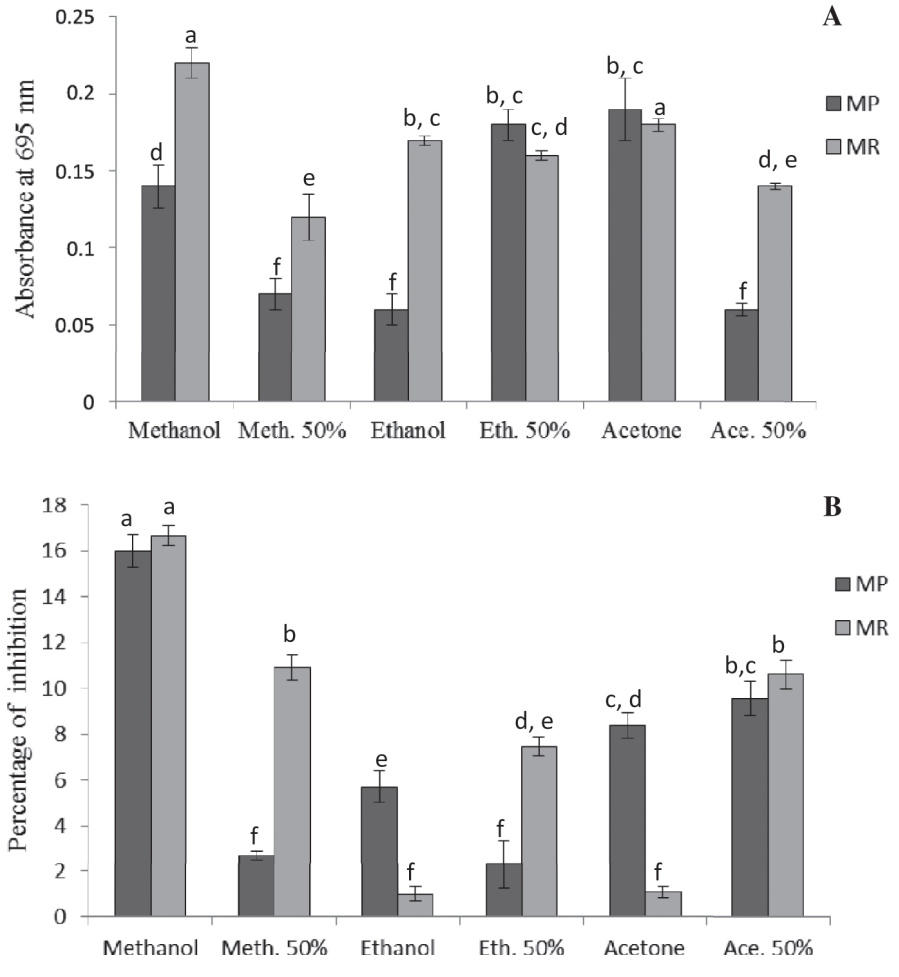

Figure 1: Total antioxidant activity (TAA) (a) and radical scavenging activity (RSA) (b) of two *Mentha* species (MP: *Mentha pulegium*; MR: *Mentha rotundifolia*) extracts. Values are expressed as the mean±SD of three determinations, values marked by the same letter are not significantly different ($p<0.05$) from each other. Meth.50 %, Eth.50 % and Ace.50 % designed respectively the mixture of methanol, acetone and ethanol with water (1:1). All the extracts were tested at the same concentration (100 µg/mL).

Maximum and minimum antioxidant capacities were observed in the acetone and ethanol extracts of MP, respectively. Whereas in case of MR methanol and 50 % methanol extract was observed. Among the extracts of *Mentha spicata* studied by Brahmi et al. [38], methanol extract exhibited high TAA.

The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids [37]. In the order to choose the adequate solvent for antioxidant capacity, the antiradical activity (DPPH test) was also evaluated. Results depicted that different leaf extracts possess a significant variability in theirs inhibitory activity against this radical, as shown in Figure 1. The results of the DPPH showed that methanol extracts of the two plants inhibited more free radicals than other solvents. The methanol extract of MP recorded the highest phenolic and flavonoids content and also had the highest antioxidant activity; however, the acetonic extract of MR that gave considerable flavonoid content recorded the lowest antioxidant activity. This suggests that the flavonoid contents of the acetone extract may be glycosylated making the flavonoids not freely available for antioxidant activity [33]. Using methanol extracts of nine *Mentha* species (*M. suaveolens, M. longifolia, M. officinalis, M. piperita, M. pulegium, M. royleana, M. arvensis, M. spicata, M. arvensis*), significant scavenging activity towards $DPPH^{\bullet}$ radical (Percentage inhibition 65 %) was noted [39]. Furthermore, methanolic extracts have previously been reported to have high RSA of *Mentha pulegium* [40] and *Mentha piperita* [41].

Correlation between TPC, TFC and TAA

Polyphenols found in plant extracts are considered the main bioactive compounds with antioxidant activity [42]. Thus, the antioxidant activity was correlated with the TPC and TFC of the extracts in order to examine if MP and MR polyphenols are responsible for it. The results in Table 2 and Figure 1 clearly indicated that the quantitative estimation of TPC, TFC, TAA and DPPH values is influenced by two variables that are extracting solvent and species of plant. Therefore, the correlation analyses between the studied parameters were analyzed within the extracts of each variable. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables [43].

Moderate to strong positive significant correlations were observed between the studied parameters, when six different solvent extractions of the two species were separately analyzed (Table 3). The correlation coefficients (r) between TPC, TFC, RSA and TAA were in a range of 0.514–1.000 ($p<0.05$) in some extracts, confirming that polyphenols are likely to contribute to antioxidant activity of these plant extracts. Similar correlation was reported by Ref. [44], they found r = 0. 989 as the correlation factor between antioxidant activity and total phenolics in some ethanolic extracts from Iranian *Mentha* species.

Table 3: Pearson's correlation coefficient (r) between antioxidant activities (obtained from total antioxidant activity (TAA) and radical scavenging activity (RSA) assays with total phenolic content (TPC) and total flavonoid content (TFC) of *Mentha pulegium* and *Mentha rotundifolia*.

Solvent	Antioxidant activity	*M. pulegium*		*M. rotundifolia*	
		TPC	TFC	TPC	TFC
Methanol	RSA	0.817[a]	0.980[a]	−0.125	−0.998
	TAA	0.916[a]	0.340	0.189	0.999[a]
Methanol. 50 %	RSA	0.995[a]	0.846[a]	0.995[a]	0.209
	TAA	−0.038	0.411	−0.500	0.803[a]
Ethanol	RSA	−0.085	0.700[a]	−0.977	−0.519
	TAA	0.988[a]	−0.665	−0.382	−0.406
Ethanol.50 %	RSA	1.000[a]	−0.840	−0.500	0.687[a]
	TAA	−0.521	−0.024	−0.427	−0.988
Acetone	RSA	1.000[a]	−0.016	−0.976	−0.247
	TAA	0.514[a]	0.849[a]	0.675[a]	0.963[a]
Acetone.50 %	RSA	0.289	0.885[a]	0.361	0.875[a]
	TAA	−0.345	0.986[a]	0.998[a]	0.725[a]

[a] Correlation is significant at $p<0.05$ level (n = 3).

On the other hand, a weak correlations existed between the RSA and TPC (r = 0.289 and 0.361 for 50 % acetone extracts from MP and MR respectively); between TAA and TPC (r = 0.189 for MR methanol extract), between RSA and TFC (r = 0. 209 for MR 50 % methanol extract) and between TAA and TFC (r = 0.340 and r = 0.411 for MP methanol and 50 % methanol extracts respectively). This implies that the phenolic compounds may not be the main component responsible for antioxidant ability of these extracts.

In addition, antioxidant activity and phenolic content were inversely related. So, negative correlations (Table 3) existed between TPC and RSA (ethanol MP extract; methanol, ethanol, acetone and 50 % ethanol MR extracts), between TPC and TAA (50 % methanol, 50 % ethanol, 50 % acetone MP extracts, 50 % methanol, 50 % ethanol and ethanol MR extracts), between TFC and RSA (50 % ethanol and acetone MP extracts, ethanol, methanol and acetone MR extracts) and between TFC and TAA (ethanol and 50 % ethanol MP and MR extracts) assays.

Stagos, Portesis [42] revealed that there was a moderate correlation of total polyphenols with deactivation of DPPH radical (r = −0.610) in *Mentha* species.

At the first glance, this negative correlation suggests that phenolic compounds are not contributed to the antioxidant activity in the extracts cited above. The antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also needs their characterization [45]. In addition, the synergism between the antioxidants in the mixture makes the antioxidant activity, not only dependent on the concentration but also on the structure and interaction between antioxidants [46].

Conclusions

To conclude, it is evident that the extraction of the antioxidant compounds from two Algerian *Mentha* species (*M. pulegium* and *M. rotundifolia*) is dependent on the extracting solvent used and the plant species. The higher TPC and TFC recoveries and RSA activity were obtained mainly from the methanol extracts. Meanwhile, for the *M. pulegium*, 50 % ethanol extract exhibited the higher total antioxidant activity.

The results reported in this paper can be useful to draw preliminary considerations on the selection of the solvent type to extract phenolic compounds from *Mentha* species. Furthermore, it can be concluded that leaves of *Mentha* used in folk medicine in different areas of Algeria can be considered as an accessible source of natural antioxidants with consequent health benefits. However, the components responsible for the antioxidative activity of their extracts are currently unclear. Therefore, it is suggested that further works could be performed on the isolation and identification of the antioxidative components in *M. pulegium and M. rotundifolia*.

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