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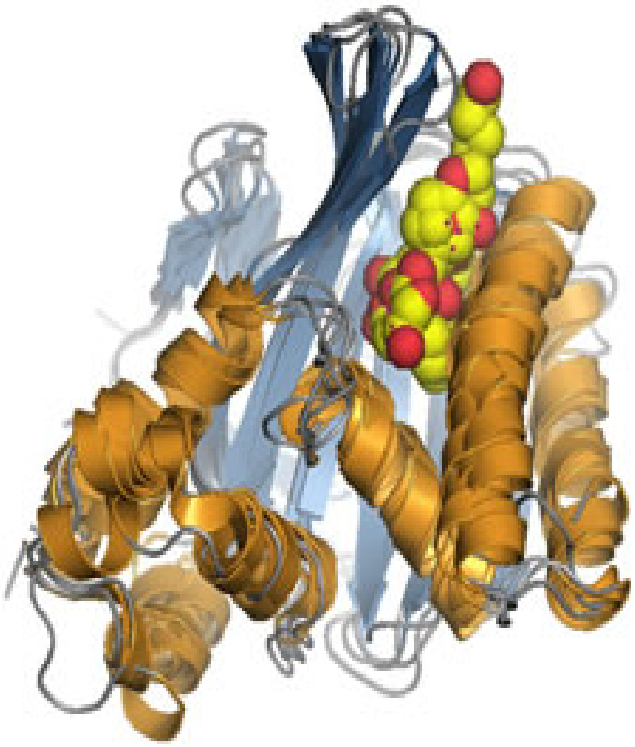

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Comparative study on the antioxidant effect of aqueous and ethanolic extracts of *Mentha pulegium* L. grown at two different locations

BRAHMI *Fatiha* [a*], BOULKBACHE-MAKHLOUF *Lila* [a], YALAOUI-GUELLAL *Drifa* [a], CHIBANE *Mohamed* [b] & MADANI *Khodir* [a]

[a] *Laboratoire 3BS, Faculté des Sciences de la Nature et de la Vie*
Université A. Mira de Bejaia, Bejaia 06000, Algérie
[b] *Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre*
Centre Universitaire de Bouira 10000, Algérie

Received: February 09, 2014; Accepted: 25 May, 2014
Corresponding author Email fatiha.brahmi@univ-bejaia.dz

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Abstract. *Aqueous and ethanolic extracts of Mentha pulegium L. (Lamiaceae) from two different locations Tizi-Ouzou and Bejaia in Algeria were examined in vitro. Phenolic compounds and antioxidant activity were analyzed. Measurement of total phenolic and total flavonoids contents of the extracts of M. pulegium were achieved by using Folin-Ciocalteu and chloride aluminium methods, respectively. The total phenolic content of water extract from Tizi-Ouzou location was found significantly higher (55.78 ± 2.78 mg GAE/g DW). The flavonoids content was also higher in the extracts from this location for both ethanolic and water extracts (2.17 ± 0.12; 2.04 ± 0.03 mg QE/g DW, respectively). Antioxidant activities were assessed by five in vitro antioxidant assays. Results showed that the two M. pulegium provenances were significantly different according to their antioxidant activity. Indeed, aqueous extract issued from Bejaia plant exhibited stronger antioxidant activity. For instance, Bejaia provenance shoots showed lower IC_{50} value of 20.35 µg/ mL for H_2O_2 test.*

Key words: Pennyroyal extracts; Antioxydant activity; Bejaia location, Tizi-Ouzou location.

1. Introduction

Mentha pulegium L. (Algerian name: Fliou) is one of the most frequently used Algerian herb, its aerial part is considered effective in treating a wide range of disorders, mainly for the treatment of flatulent dyspepsia and intestinal colic due to its carminative and antispasmodic properties (Dellile, 2007). Its essential oil and dry parts have been traditionally used in medicine (digestive, liver and gallbladder disorders, amenorrhea, gout, colds, increased micturition, skin diseases and abortifacient), gastronomy (culinary herb), aromatherapy and cosmetics. Recent research reported its antioxidant and antimicrobial properties (Teixera et al., 2012).

The occurrence of at least some of mentioned above diseases as well as aging processes may result from oxidative stress leading to a variety of alterations within the human organism caused by reactive oxygen species (ROS). ROS is a collective term used to include both the

oxygen radicals and some non-radical reactive derivatives of oxygen (Mata et al., 2007; Nickavar et al., 2008).

Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate them. The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an overabundance of ROS from an environmental or behavioral stressor (Mata et al., 2007). If not controlled properly, the excess of ROS can lead to damage of cellular lipids, proteins or DNA, impairing their normal function. The organism defends itself against ROS by engaging several enzymatic systems and endogenous antioxidants. This natural defense is enhanced by antioxidants delivered with diet (Nickavar et al., 2008; Senevirathne et al., 2006).

Polyphenols are widely distributed in plants and phenolic antioxidants have been found to act as free radical scavengers as well as metal chelators (Senevirathne et al., 2006). Many species of the Lamiaceae family are known for their antioxidant effect (Matkawski and Piotrowska, 2006), among them pennyroyal (*Mentha pulegium* L.). Although some studies have been conducted to determine the antioxidant activity, the content of polyphenols and flavonoids as well as other antioxidants in mints (Mata et al., 2007; Nickavar et al., 2008; Teixera et al., 2012) is still not well known and their content depend on many factors.

It is well documented that the quantity and quality of herbs grown in different regions are significantly influenced by environmental and growing conditions (Carmona et al., 2007). Moreover, many factors contribute to the efficiency of the solvent extraction process especially the type of the solvent (Chirinos et al., 2007). Therefore, plants of *M. pulegium* were collected at two different locations in Est Algeria called petite Kabylie (Bejaia) and grande Kabylie (Tizi-Ouzou Mountains). Subsequently, aqueous and ethanolic extracts were produced from plants of both locations.

So, the purpose of this study was to compare the total phenolics as well as the flavonoids content and the antioxidant activity in different pennyroyal extracts. It seems, that such data will be of great value, as current knowledge indicates that (i) antioxidant activity and phenolic of plants varied between locations from contrasting climates; (ii) the type of the solvent have also influence on the efficiency of total phenols, flavonoids and the antioxidant properties of extracts.

2. Materials and methods

2.1. Plant sampling and extraction

Shoots (leaves and stems) were harvested from two regions from June to August 2009: Bejaia (Est of Algeria, humid bioclimatic stage, Lat: 36° 43' N, Long: 5° 04' E, altitude of 2 m,) and Tizi-Ouzou (Est of Algeria, superior semi-arid bioclimatic stage, Lat: 36° 42' N, Long: 4° 03' E, altitude of 189 m) differing in their climatic conditions (Table 1).

Table 1. Climatic characteristics (temperature and precipitations) of the studied localities where *M. pulegium* shoots were harvested. Jun: June, Jul: July, and Aug: August.

Locality	Average temperature (°C)			Light hours (h)			Precipitations (mm)		
	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug
Bejaia	23.0	27.0	27. 0	348.0	321.0	300.0	1.0	4.0	15.0
Tizi-Ouzou	25.6	30.1	28.9	-	-	-	0.0	3.0	7.0

Plants were identified at the laboratory of botany (University of Bejaia) and later validated by Professor J. Lejoly in the Laboratory of Systematical Botany and Phytosociology, Free University of Brussels (ULB), Belgium. A voucher specimen [BR 000000 6946043] was deposited at the Herbarium of the National Botanical Garden of Meise (Belgium). The collected shoots were rinsed and dried away from direct sunlight before being ground in a Mettler (Kika Labortechnik, Staufen, Germany). Ethanolic and aqueous extracts were obtained by magnetic stirring of 2.5 g dry powder with 25 mL pure ethanol or water for 24 h. Extracts were filtered through a Whatman No 1 filter paper, evaporated to dryness and stored at 4 °C until analyses.

2.3. Colorimetric quantification of phenolics

2.3.1. Total polyphenol content

The amount of total phenolics in shoot extracts was determined using the method described by Singleton and Rossi (1965) using a calibration curve of gallic acid. To 100 µL of shoot extracts, 500 µL of Folin-Ciocalteu reagent and 6 mL of distilled water were added. The mixture was shaken before adding 1500 µL of Na_2CO_3 (20% w/v) and adjusting with distilled water to 10 mL. After incubation for 60 min in the dark, the absorbance was read at 760 nm. Results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE/ g DW).

2.3.2. Total flavonoid content

Quantification of flavonoids was based on the method described by Bahorun et al. (1996). An aliquot of extract or standard (quercetin) was added to 1.5 mL $AlCl_3$ solution (2%). After 15 min of incubation at room temperature, absorbance was determined at 430 nm and flavonoids concentration was calculated according to the equation obtained from quercetin graph, and were expressed as mg quercetin equivalents per gram dry weight (mg QE/ gDW).

2.4. Determination of antioxidant activity

2.4.1. Phosphomolybdenum assay

The total antioxidant activity (TAA) of samples and standards was evaluated by the green phosphomolybdenum complex according to the method of Prieto et al. (1999). An aliquot of 100 µl of sample solution was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled at room temperature, the absorbance of the mixture was measured at 695 nm against a blank. Increased absorbance indicates increased TAA. The TAA values have been expressed as IC_{50} (µg/mL) that is the effective concentration at which the absorbance was 0.5 and was obtained by interpolation from linear regression analysis. All samples were analyzed in triplicates.

2.4.2. Reducing power

The reducing power of the extracts was evaluated according to the protocol of Oyaizu (1986). 1 mL of different concentrations of the samples was mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1%). The mixture was incubated at 50 ◦C for 20 min. Trichloroacetic acid (TCA) (1 mL, 10%) was added to the solution which was then centrifuged for 10 min at 3000 g. The supernatant was gathered and mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm and compared to the standards (BHA and α-tocopherol). IC_{50} value (µg/ mL) is the effective concentration at which the absorbance was 0.5 for reducing power. It was obtained from linear regression analysis. All samples were analyzed in triplicates.

2.4.3. Stable free radical scavenging activity using DPPH° method

The antioxidant activities of *M. pulegium* extracts were measured in terms of hydrogen donating or radical scavenging ability, using the DPPH method (Brand-Williams et al., 1995). A solution (0.1 mL) of the sample extracts at various concentrations was added to 2.0 mL (0.1 mM) of DPPH solution. The solution was incubated at room temperature for 30 min and the decrease in absorbance at 517 nm was determined at the end of incubation period with a spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the samples and was calculated using the following equation:

% radical scavenging activity = [(Control OD – Sample OD)/ Control OD] * 100

The radical scavenging activity was expressed as IC_{50} (µg/ mL), the extract dose required to cause a 50% inhibition. A low IC_{50} value corresponds to a high antioxidant activity of the plant extracts. All samples were analyzed in triplicates

2.4.4. Determination of hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Göktürk Baydar et al. (2007). A 2 mM solution of hydrogen peroxide was prepared in phosphate buffer (pH = 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Extracts (50, 100, 200, 300 and 400 µg/mL) were added to 0.6 mL of hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.

The percent of scavenging of hydrogen peroxide of extracts was calculated according to the formula: % scavenged $H_2O_2 = [(A_0 - A1)/A_0] \times 100$;

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the samples of pennyroyal extracts. All determinations were done in triplicate.

2.4.5. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was based on the methodology reported by Teixera et al. (2012). Briefly, FRAP reagent was prepared by combining 1mM TPTZ with 2mM ferric chloride in 0.25M sodium acetate (pH 3.6). The sample (0.2 mL) was mixed with FRAP reagent (1.8 mL), stood for 4min at room temperature and the absorbance was determined at 593 nm.

The standard was prepared from ferrous sulphate that reacted with the TPTZ reagent, following the same procedure as with samples. The absorbance was plotted against ferrous ion concentration (0.2–1.0 mM Fe^{2+}), and FRAP values were expressed as mmol Fe^{2+} per g of sample.

2.5. Statistical analysis

All experiments were conducted in triplicates and results are expressed as mean ± standard deviation (SD). Data were subjected to analysis of variance, and means were compared by least significant difference (LSD). Differences at $P < 0.05$ were considered to be significant.

3. Results

3.1. Extraction yields, total phenolics and flavonoids contents of extracts

The yields, total phenolic and flavonoids contents of *M. pulegium* extracts from two different locations and with two solvents are given in Table 2. Yields of the extracts ranged

from 4.65% to 24.06%, the total phenolic contents varied from 8.03 to 55.78 mg GAE/g DW and the total flavonoids varied from 1.62 to 2.17 mg QE/g DW.

Table 2. Yield, total phenolics and total flavonoids content of *M. pulegium* extracts

Extract	Yield (%)	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
MPT-Water	20.06	55.78 ± 2.78^{a}	2.04 ± 0.03^{a}
MPT-EthOH	4.65	43.69 ± 0.89^{b}	2.17 ± 0.12^{a}
MPB-Water	24.06	11.08 ± 0.33^{c}	1.87 ± 0.07^{b}
MPBE-EthOH	5.20	7.81 ± 019^{d}	1.63 ± 0.05^{c}

MPT, *Mentha pulegium* from Tizi-Ouzou; MPB, *Mentha pulegium* from Bejaia; EthOH, Ethanol. Values followed by different superscripts (a–c) in the rows are significantly different at $p < 0.05$ (means of the replicates).

3.2. Antioxidant activity assays

3.2.1. Phosphomolybdate assay

In the presence of extracts, Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum V complex, which shows a maximum absorbance at 700 nm (Sahreen et al. 2010). Activity of all samples was dose dependent (Figure 1). The antioxidant capacity of different extracts of pennyroyal shoots can be ranked in the order of water from Tizi-Ouzou (WT) > water from Bejaia (WB) > ethanol from Bejaia (EB) > ethanol from Tizi-Ouzou (ET) extracts. The IC_{50} values of the antioxidant capacity for the WT, WB and EB and extracts were 252.01 ± 8.36', 269. 99 ± 0.16 and 456.09 ± 8.32 µg/mL, respectively, while for ET extract it was > 500 µg/mL (Table 3). However, the total antioxidant of all the extracts were found to be much lower ($P < 0.05$) when compared to standards.

3.2.2. Ferric reducing antioxidant power

FRAP assay is based on the reduction of Fe^{3+}–TPTZ complex, to the blue colored ferrous form, with an increase in absorbance at 593 nm (Yang et al., 2010). The antioxidant activity of *M. pulegium* measured by the FRAP method ranged between 1.76 and 2.03 mmol of Fe^{2+} per g of dry weigh (Table. 3). The water extract from Bejaia had statistically the highest antioxidant activity, followed by water and ethanolic extracts from Tizi-Ouzou and ethanolic extract from Bejaia.

3.2.3. Reducing power

The presence of antioxidants in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} form and Fe^{2+} can be monitored by measurement of the formation of Perl's Prussian blue at 700 nm (Zhu et al., 2011). All the extracts showed some degree of electron-donating capacity in a linear concentration-dependent manner (Figure 2).
The ferric reducing power of *M. pulegium* revealed that the water extracts had statistically the highest antioxidant activity, followed by the ethanolic extract from Bejaia, and the ethanolic extract from Tizi-Ouzou (IC_{50} varying between 235.17 and 423.98 µg/mL) (Table 3).

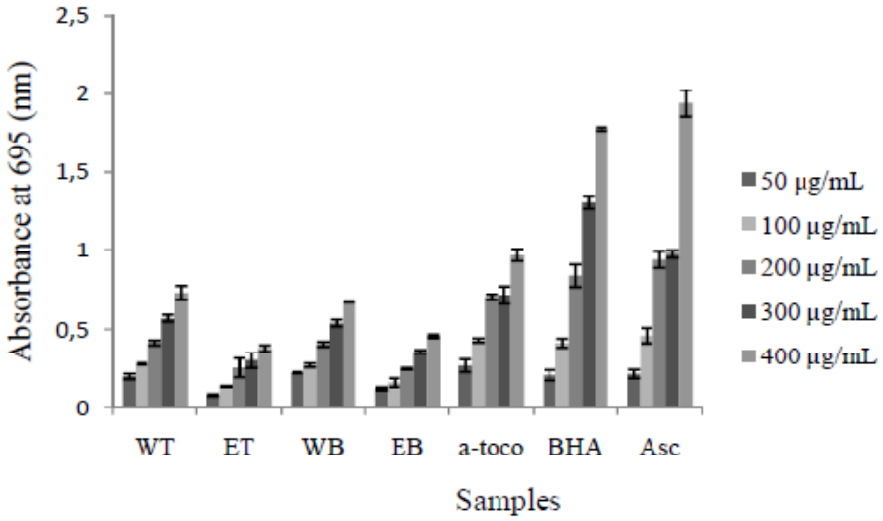

Figure 1. Comparison of total antioxidant activity from pennyroyal extracts from two different locations and using two solvents (WT, water-Tizi-Ouzou; ET, ethanol Tizi-Ouzou; WB, water Bejaia,; EB, ethanol Bejaia) and standards (α-toco, α-tocopherol; BHA, butylated hydroxyanisole; Asc, ascorbic acid). Different letters above the bars for the same concentration indicates significant differences among means of treatments ($P < 0.05$).

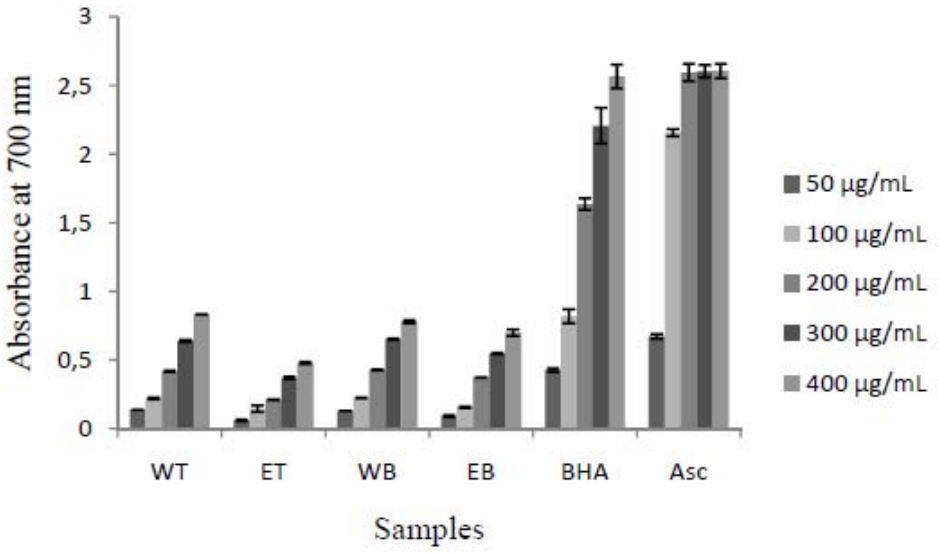

Figure 2. Comparison of reducing power capacity from pennyroyal extracts from two different locations and using two solvents (WT, water-Tizi-Ouzou; ET, ethanol Tizi-Ouzou; WB, water Bejaia,; EB, ethanol Bejaia) and standards (BHA, butylated hydroxyanisole; Asc, ascorbic acid). Different letters above the bars for the same concentration indicates significant differences among means of treatments ($P < 0.05$).

Table 3. Antioxidant effect (IC_{50}) on DPPH, H_2O_2 radicals, total antioxidant capacity, reducing power, and Ferric reducing antioxidant power of ethanolic and aqueous extracts from two locations of pennyroyal shoots.

Sample	IC_{50} µg/mL				
	Phospho-molybdate assay	Reducing power	Scavenging ability on hydrogen peroxide	Scavenging ability on DPPH radicals	FRAP (µM/g sample)
WT	252.01 ± 8.36^{b}	235. 17 ± 2.45^{c}	234.87 ± 2. 08^{c}	364.89 ± 3.56^{c}	1.87 ± 0.11^{b}
ET	511.87 ± 0.66^{d}	423. 98 ± 3.11^{b}	178. 44 ± 2.36^{b}	484.84 ± 2.38^{d}	1.86 ± 0.14^{b}
WB	269. 99 ± 0.16^{b}	239. 79 ± 2.04^{c}	232.89± 1. 74^{c}	331.65 ± 3.41^{a}	2.03± 0.06^{a}
EB	456. 09 ± 8.32^{c}	280. 81± 4.90^{d}	20.35 ± 1.92^{a}	348.84 ± 1.92^{b}	1.76 ± 0.03^{c}
BHA	119.64 ± 3.51^{a}	51. 14 ± 1.64^{b}	-	-	-
Ascorbic acid	126. 38 ± 4.50^{a}	31. 66 ± 1.19^{a}	-	-	-

Each value in the table is represented as mean ± SD (n = 3). Means not sharing the same letter are significantly different (LSD) at $P < 0.05$ probability level in each column. BHA: butylated hydroxyanisole, DPPH: 1,1-diphenylpicryl-hydrazyl, BE: Béjaia extract; TE: Tizi-Ouzou extract, WE: Water extract.

3.2.4. DPPH radical scavenging activity

DPPH° radical is a stable organic free radical with an absorption band at 517 nm. It loses this absorption on accepting an electron or a free radical species, which results in a visually noticeable discolouration from purple to yellow (Zhu et al., 2011). In this study, all extracts are a source of radical scavenging activity, this activity changed with the solvent used to prepare the extract and from location to another. These extracts showed DPPH scavenging activities in a concentration-dependent manner whose profiles varied among the different extracts (Figure 3). The radical scavenging activities from Bejaia extracts were considerably better than that of the extracts from Tizi-Ouzou (Table 3).

3.2. 5. Hydrogen peroxide radical scavenging activity

The measurement of hydrogen peroxide scavenging activity can be one of the useful methods determining the ability of antioxidants to decrease the level of prooxidants such as hydrogen peroxide (Guktuk Bayder, 2007). Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells, since it may give rise to hydroxyl radicals inside the cell (Sahreen et al., 2010). Extracts from *M. pulegium* were capable of scavenging hydrogen peroxide in a concentration dependent manner (50 – 400 µg/mL) (Figure 4). As compared with the IC_{50} values, the hydrogen peroxide scavenging activities of ethanolic from Bejaia (20.35 ± 1.92 µg/mL) and Tizi-Ouzou (178. 44 ± 2.36 µg/mL) extracts were more ($P < 0.05$) effective than that of water extracts (232.89 ± 1.74; 234.87 ± 2. 08 µg/mL, respectively, from Bejaia and Tizi-Ouzou locations) .

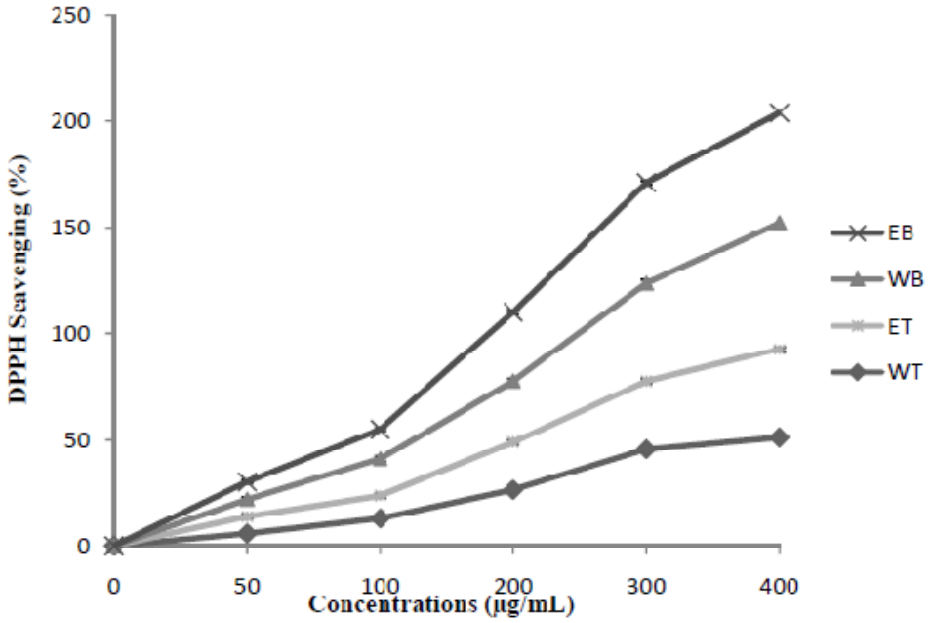

Figure 3. DPPH radical scavenging activity of different extracts from the extracts of pennyroyal shoots by two solvents and from two locations.
Each value represents a mean ± SD (n = 3); EB, ethanol extract from Bejaia location; WB, water extract from Bejaia location; ET, ethanolic extract from Tizi-Ouzou location; WT, water extract from Tizi-Ouzou location

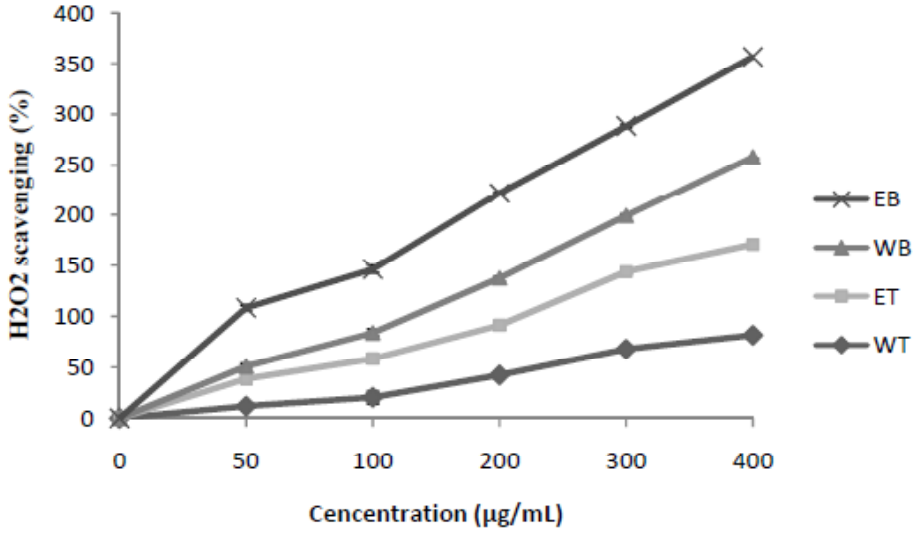

Figure 4. Hydrogen peroxide radical scavenging activity of different extracts from the extracts of pennyroyal shoots by two solvents and from two locations.
Each value represents a mean ± SD (n = 3); EB, ethanol extract from Bejaia location; WB, water extract from Bejaia location; ET, ethanolic extract from Tizi-Ouzou location; WT, water extract from Tizi-Ouzou location

4. Discussion

Extraction yield of Bejaia plant is better than that of Tizi-Ouzou one. Otherwise, yield of water extract is better than that of ethanol one, for both plants. A similar trend was observed in a study of the aqueous and ethanolic extracts of the defatted wheat germ (Zhu et al., 2011). They found the yields ranged from 5.16% for the ethanol extract to 48.03% for the aqueous extract which might be attributable to the higher solubility of proteins and carbohydrates in water than in ethanol.

However, the content of total phenolics was systematically lower in Bejaia extracts compared to that of Tizi-Ouzou. We also noted that water extracts showed the best total phenolics content for the two regions. This could be explained by the possible formation of complexes by a part of the phenolic compounds with carbohydrates and proteins, which are more extractable in water than in ethanol (Zhu et al., 2011).

Teixera et al. (2012) found that hot water extract of *M. pulegium* showed the highest phenol content. In contrast, Mata et al. (2007) observed that total phenol contents of *M. pulegium* was higher in ethanolic extract (71.7 mg pyrogallol per g of sample) than in hot water extract (57.9 mg pyrogallol per g of sample).

The amount of total phenolic contents of water extract from Tizi-Ouzou in the present study was almost similar to the amount reported by Mata et al. (2007) (57.9 ± 1.6 mg/g DW). These values were rather low compared to those obtained with hot water extracts of other *Mentha* species, where values varied between 128.1 to 230.8 mg gallic acid per g of sample (Dorman et al., 2003).

While the highest extract yield was obtained from the BE extracted with water, the maximum total flavonoids content was found in the TE extracted with ethanol. Brahmi et al. (2012) reported that different amounts of total flavonoids contents were found in different solvent extracts of mint (*M. spicata*). On the other hand, not only the total phenolic contents but also the total flavonoids contents were low in Bejaia extracts. Such coincidences might be caused by the specific ‘‘terrior” factor. Plant species have inherent physiological differences, as a result of interactions with their environment (Taulavuori et al., 2010). According to Li et al. (2011), the accumulation of phenolic compounds in grape berries is strongly affected by ‘‘terroir” factors. A part probable edaphic difference, it seems that rainfall scarcity and long light exposure may be involved in the activation of phenol biosynthesis (Naczk and Shahidi, 2006). Besides, previous researches showed that light, water deficits and higher temperature differences between daytime and nighttime could up-regulate the gene expression related to flavonoids metabolism, and thus significantly increase the contents of flavonoids. Infertile soil, rather than fertile ones, provides with more composite and content of inorganic ions, activating flavonoids synthesis (Li et al, 2011).

Indeed, Tizi-Ouzou is located in mountains zones with altitudes of 189 m above sea level. This plateau provides with a warm-arid climate, a big temperature difference between daytime and night time, an annual rainfall of 974.1 mm which could explain the high levels of phenolic compounds in pennyroyal from this location. But, Bejaia is located on the plain at the altitude of 2 m with a cool-warm, semi-humid climate, an annual rainfall of 1107 mm. Regardless of the mechanisms involved, the ‘‘terroir” factor is believed to participate in the regulation of the phenolic compound biosynthesis in pennyroyal. Accordingly, phenolic contents were higher in plants growing in more stressful climatic conditions (Tizi-Ouzou) than those coming from more appropriate environment (Bejaia).

Considering antioxidant activity, results revealed that Bejaia plants exhibited higher capacity to neutralize ROS than Tizi-Ouzou ones in almost all cases. Besides, water extracts

were more effective than ethanolic ones. Since the two provenances derived from the same mother plants, differences between them are mainly due to environmental conditions or to the nature of the solvent used. So, antioxidant activity changed with the solvent used to prepare the extract (Brahmi et al. 2012). Antioxidant studies with the pennyroyal shoots ethanol and water extracts have already been reported. Previous studies with hot water extracts of *Mentha* species (*M. pulegium* not included) showed slightly higher ferric reducing power (Dorman et *al*., 2003). Nickavar et al. (2008) reported that ethanolic extract of this plant exhibited higher antioxidant activity (DPPH•) (IC_{50} = 17.92 µg mL^{-1}), compared to the values obtained in our study.

Yet, the best results were obtained with water extracts of the pennyroyal (IC_{50} = 8.9 ± 0.2 µg/ mL) (Mata et al. 2007). The ferric reducing power of *M. pulegium* revealed that the hot water extract had statistically the highest antioxidant activity, followed by the ethanolic extract (Texeira et *al*., 2012).The DPPH assay also identified *M. pulegium* hot water extract (IC_{50} = 16.3 ± 0.4µg/ mL) as having statistically the highest free radical scavenging activity, followed by ethanolic extract (Texeira et al., 2012).

On the other hand, these differences may be reflected by the presence of various chemical compounds that provide information regarding the ecotype conditions (Taulavuori et al., 2010). Variability depending on climate conditions was observed by Ozgen et al. (2009) when comparing the reducing power of 14 *Moruss nigra* accessions.

Previous reports on plant extracts suggested that abiotic constraints enhance antioxidant activity as a response to the oxidative stress generated in these aggressive environments (Falleh et al., 2012). So, exposure to environmental stress, such as water deficit often augments the production of reactive oxygen species (ROS), which has direct and indirect effects on the synthesis of antioxidants and on secondary metabolism (Ksouri et al., 2007). With this respect, our results corroborate negatively those of Falleh et al. (2012) studying the edible halophyte from two contrasting climatic regions (Djerba and Monastir sampled from arid and superior semi-arid bioclimatic stages, respectively).

It was found that phenolics were the main antioxidant components and its total content was directly proportional to the antioxidant activity (Zhu et al., 2011). But in this paper, the relationship between the total phenolic content and the antioxidant activities of the extracts were complex. Several reasons could be provided for this observation: (i) the different extraction solvent and the origin of the plants resulted in the differences of the extracts in their compositions, and consequently their antioxidant activities (Zhu et al., 2011); (ii) according to Jayaprakasha et al. (2008) polyphenolic and antioxidant index is a combined measure of the quality and quantity of antioxidants in vegetables. Thereby, regarding the effect of location, probably, the quality not the quantity of the compounds had an important potential to imbalance the generation of ROS by improving their scavenging system constituents. So, this activity would depend on the chemical structure of phenolic compounds and the availability of phenolic hydroxyl groups which have the capacity to donate their electron or hydrogen thereby forming stable end product (Jayaprakasha et al., 2008); (iii) the antioxidant assays used were based on different mechanisms and conditions, so that they may present differing results, each only partially reflecting the antioxidant activity (Arabshahi-Delouee and Urooj, 2007); (iv) the Folin-Ciocalteu assay to measure the total phenolic content of extracts could be disturbed by other components (Prior et al., 2005). It was also thought that all other soluble compounds present in the extracts, including proteins, peptides, polysaccharides, and pigments, could be responsible for the antioxidant activity partly (Prior et al., 2005; Zhu et al., 2011).

5. Conclusion

In summary, this study demonstrated the implication of phenolic quantity and the antioxidant capacity of *M. pulegium* in the adaptation towards its environment (location). Indeed, environmental conditions influence the antioxidant potential related to the phenolic composition of *M. pulegium* shoots. Since there are safety concerns associated with the use of water and ethanol we have used them for extraction of phenolic compounds from pennyroyal. This extraction solvent affected the levels of total phenolics, flavonoids and antioxidant contents extracted from pennyroyal from the two different Algerian locations.
Therefore, this study strongly supports the idea that polyphenols play a significant physiological role in *M. pulegium* adaptation to environmental conditions, particularly to enhance the production of these metabolites.

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References

Arabshahi-Delouee, S. & Urooj, A. 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chemistry* 102: 1233–1240.

Bahorun, T ., Gressier, B ., Trotin, F ., Brunet, C., Dinet, T ., Luyckx, M., Vasseur, J., Gazin, M., Cazin, J.G. & Pinkas M. 1996. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimtte-Forschung Drug Research* 46: 1086 – 1108.

Brahmi, F., Madani, K., Dahmoune, F ., Rahmani, T., Bousbaa, K., Oukmanou, S. & Chibane, M. 2012. Optimisation of solvent extraction of antioxidants (phenolic compounds) from Algerian mint *(Mentha spicata* L.). *Pharmacognosy Communications* 2: 72-86.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. u. –Technolology* 28: 25-30.

Carmona, M., Sánchez, A.M., Ferreres, F., Zalacain, A., Tomás-Barberán, F. & Alonso, G.L. 2007. Identification of the flavonoid fraction in saffron spice by LC/DAD/MS/MS: comparative study of samples from different geographical origins. *Food Chemistry* 100: 445–450.

Chirinos , R ., Rogez, H ., Campos, D ., Pedreschi, R & Larondelle, Y. 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruız & Pavon) tubers. *Separation Purification Technology* 55 : 217 – 225.

Delille, L . 2007. Les plantes médicinales d'Algérie . ed Berti , Alger. 249 p .

Dorman, H.J.D., Kosar, M., Kahlos, K., Holm, Y. & Hiltunen, R. 2003. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *Journal of Agricultural and Food Chemistry* 51: 4563-4569.

Falleh, H., Ksouri, R., Boulaaba, M., Guyot, S., Abdelly, C. & Magné, C. 2012. Phenolic nature, occurrence and polymerization degree as marker of environmental adaptation in the edible halophyte Mesembryanthemum edule. *South African Journal of Botany* 79: 117–124

Jayaprakasha, G.K., Girennavar, B. & Patil, B. S. 2008. Radical scavenging activities of rio red grapefruits and sour orange fruit extracts in different *in vitro* model systems. *Bioresource Technology* 99: 4484 – 4494.

Gokturk Baydar, N., Ozkan, G. & Yasar S., 2007. Evaluation of the antiradical and antioxidant potential of grape extracts. *Food Control* 18: 1131–1136.

Mata, A.T, Proença, C., Ferreira, A.R, Serralheiro, M.L.M., Nogueira, J.M.F. & Araújo, M.E.M., 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices . *Food Chemistry* 103: 778 – 786.

Matkowski, A. & Piotrowska, M. 2006. Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. *Fitoterapia* 77: 346 – 353.

Nickavar, B., Alinaghi, A. & Kamalinejad, M. 2008. Evaluation of the antioxidant properties of five *Mentha* species. *Iranian Journal* of Pharmaceutical *Research* 7: 203-209.

Oyaizu, M. 1986. Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. *Nippon Shokulin Kogyo Gakkaishi* 35: 771–775.

Ozgen, M., Serçe, S. and Kaya, C. 2009. Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *Morus rubra* fruits. *Scientia Horticulturae* 119: 275 –279.

Prieto, P., Pineda, M. and Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1. *Analytical Biochemistry* 269: 337–341.

Prior, R.L., Wu, X. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53: 4290–4302.

Li, Z., Pan, Q., Jin, Z., Mu, L. & Duan, C., 2011. Comparison on phenolic compounds in *Vitis vinifera* cv. Cabernet Sauvignon wines from five wine-growing regions in China. *Food Chemistry* 125: 77–83.

Naczk, M. & Shahidi, F. 2006., Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *Journal* of *Pharmaceutical* and *Biomedical Analysis* 41: 1523–1542.

Ksouri, R., Megdiche, W., Debez, A., Falleh, H., Grignon, C. & Abdelly, C. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritime. *Plant Physiology* and *Biochemistry* 45: 244 – 249.

Sahreen, S., Khan, M.R. & Khan, R.A. 2010. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food Chemistry* 122: 1205 – 1211.

Senevirathne, M., Kim, S-H., Siriwardhana, N., Ha, J-H., Lee, K-W. & Jeon, Y-J. 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Science and Technology International* 12: 27–38.

Singleton, V.L. & Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144 – 158.

Taulavuori, E., Taulavuori, K., Niinimaa, A. & Laine, K. 2010. Effect of ecotype and latitude on growth, frost hardiness, and oxidative stress of south to north transplanted scots pine seedlings. *Journal of Forestry Research*. doi:10.1155/2010/162084.

Teixeira, B., Marques, A., Ramos, C., Batista, I., Serrano, C., Matos, O., Neng, N.R., Nogueira, J. M. F., Saraiva, J.A. & Nunes, M. L. 2012. European pennyroyal (*Mentha pulegium)* from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. *Industrial Crops and Products* 36: 81–87.

Yang, Y., Yue, Y., Runwei, Y. & Guolin, Z. 2010. Cytotoxic, apoptotic and antioxidant activity of the essential oil of Amomum tsao-ko. *Bioresource Technology* 101: 4205 –4211.

Zhu, K.X., Lian, C.X., Guo, X.N., Peng, W. & Zhou, H.M. 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. *Food Chemistry* 126: 1122 – 1126.

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