

Original Research Article

Total phenolic and flavonoid contents of *Mentha spicata* leaves aqueous extracts in different regions of Algeria and their antioxidant, and antidiabetic activities

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Abstract

Purpose: To evaluate the total phenolic and flavonoid contents of *Mentha spicata* (MS) leaves aqueous extracts obtained from different regions (El-Oued, Tebessa and El-Tarf) of Algeria, as well as their in vitro antioxidant and antidiabetic activities.

Methods: Aqueous extracts were obtained from the air-dried leaves of MS by maceration followed by filtration and evaporation using a rotary evaporator. Folin-Ciocalteu and Aluminium chloride colorimetric techniques were used to determine the total phenolic and flavonoid contents, respectively. The radical DPPH, ABTS, and FRAP tests were used to determine their antioxidant activities, while the in vitro inhibition percentages of α -amylase and α -glucosidase enzymes were used to assess the anti-diabetic activity.

Results: The El-Oued's extract exhibited the highest total phenolic content (108.94 mg GAE/g dry extract (DE)), while the highest total flavonoid content (0.039 mg QE/g DE) was found in El-Tarf's extract. The radical DPPH and FRAP scavenging activity of the El-Oued extract exhibited the highest inhibition activities ($IC_{50} = 102.5$ and $289.5 \mu\text{g/mL}$), respectively, while the ABTS inhibition activity of the El-Tarf extract exhibited a maximum IC_{50} value of $111 \pm 2.8 \mu\text{g/mL}$. The MS extract of the El-Oued region had the highest α -amylase and α -glucosidase enzyme activities with IC_{50} values of 121.4 and 216.9 $\mu\text{g/mL}$, respectively.

Conclusion: The leaves of *Mentha spicata* exhibit high phenolic and flavonoid contents, along with significant antioxidant and antidiabetic properties. This study reveals that *Mentha spicata* flavonoid and phenolic contents as well as other properties vary by region.

Keywords: *Mentha spicata*, Antidiabetic, Antioxidant, α -Amylase, α -Glucosidase

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INTRODUCTION

The term, "alternative medicine", refers to all forms of unconventional medicine. It utilizes

conventional and alternative approaches focused on medicinal plant treatments [1]. Various plant parts, such as stems and leaves, are employed to treat pains. These plant parts are rich in

bioactive chemicals most of which are secondary metabolites [2]. Plant essential oils are also employed in medicinal preparations and food industries to improve taste and flavour [3]. *Mentha* is a perennial herb of the Lamiaceae family that has 25 - 30 species. It is grown across temperate Eurasia, Australia, and South Africa [4]. *Mentha spicata* L. is the most well-known specie in the genus *Mentha*. It is a creeping rhizomatous perennial herb well-known for its medicinal and aromatic properties [3]. It is used regularly as a flavour in cooking, chewing gum, toothpaste, mouthwashes and cosmetics [5] and also as an antiseptic, antifungal and antimicrobial and antioxidant agent [6]. It is widely used traditionally as a tea and spice to flavour foods, dishes and drinks. *Mentha spicata* L. is planted for commercial purposes all over the world, including Algeria [7], which, due to its vast geographical area, has a wide range of climatic and terrestrial variabilities.

Previous research demonstrated that the amount of secondary metabolites and biological activities in plants depend on environmental conditions [5]. These conditions include the harvesting season, soil fertilization, and geographic location which can impact on the amount of secondary metabolites [8]. As a result of seasonal fluctuations in temperature and precipitation, bioactive components in medicinal plants vary considerably from one season to the other [4]. In this context, temperature and the region of cultivation play a major role in the phytochemistry and secondary metabolite activity of the plant [9]. Therefore, the present study aimed to compare the total phenolic and flavonoid contents as well as their antioxidant and antidiabetic activities of the aqueous extracts of *Mentha spicata* L. leaves collected from three different regions of Algeria (El-Oued, El-Tarf, Tebessa).

EXPERIMENTAL

Chemicals

Ethanol, aluminium chloride, gallic acid, ascorbic acid, Folin-Ciocalteu, DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate), ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), TPTZ (2,4,6-Tris(2-pyridyl)-1,3,5-triazine), Potassium persulfate ($K_2S_2O_8$), trichloroacetic acid, potassium ferricyanide and Iron (III) chloride were obtained from Sigma Aldrich, USA and used without further purification.

Plant sampling and extract preparation

Mentha spicata leaves were collected in the spring from three regions of Algeria namely El-

Oued, Tebessa and El-Tarf. El-Oued region is located in the south (coordinates, 33.3678 °N, 6.8517 °E), Tebessa region (35.4011 °N, 8.1173 °E) in the continental interior, and the El-Tarf region (36.7577 °N, 8.3076 °E) on the coast. Leaf samples were washed in pipe borne water to eliminate trash and dust as much as possible. The excess water was removed by gently pressing with paper towels. The samples were dried by keeping them in open air under shade for three weeks. The dried sample was milled to semi powder form using mortar and pestle, passed through a 100-mesh sieve and stored in air tight polythene bags for further analysis.

Extracts were obtained using distilled water. Ten grams of grinded material was macerated with 100 mL of water using magnetic stirrer for 12 h at 25 ± 2 °C. The resulting mixture was concentrated using a rotary vacuum evaporator after being filtered via Whatman 4 filter paper. Then, the concentrate lyophilized and weighed to determine the yield of extraction. The obtained extracts were stored in brown bottles at 4 °C until further use.

Assay of total phenolic and total flavonoid contents

The total contents of phenols (TPC) and flavonoids (TFC) were determined using the Folin-Ciocalteu and the aluminum chloride colorimetric methods, respectively [9]. The TPC was expressed in mg of gallic acid equivalent/g of dry extract (mg GAE/g DE) while the TFC was expressed as mg quercetin equivalent/g of dry extract (mg QE/g DE).

Biological activities

DPPH radical scavenging activity

The potential scavenging activity of *Mentha* extracts was determined against DPPH free radical. The extract was diluted in ethanol at various concentrations ranging from 50 to 400 µg/mL, and 1 mL of each diluted extract was added to 0.5 mL of a 20 mg/L ethanolic solution containing DPPH. The finished mixture was left in the dark at room temperature for 30 min. At 517 nm, the mixture's absorbance was measured. The antiradical activity was represented as IC₅₀ (µg/mL) [6].

Eq 1 was used to determine the capacity to scavenge DPPH radical which served as a measure of inhibitory activity/inhibition (H).

$$H (\%) = \{(Ac - As)/Ac\}100 \dots\dots (1)$$

where A_s is the sample absorbance and A_c is the control absorbance

ABTS radical cation decolorization assay

The extracts were also tested using a colorimetric technique for their capacity to scavenge the ABTS radical cation, with Vitamin C serving as a positive control. The method was described in a previous study by Abdel-Hady *et al* [10]. The solutions obtained were incubated in the dark for 6 min and then the absorbance was measured at 734 nm. The ABTS scavenging activity was determined using the same formula that was used with for DPPH test (equation 1).

Ferric reducing ability power assay

The ferric reducing power (FRAP) test was used to assess the FRAP of the extracts following the method earlier described by Brahmi *et al* [7]. The effect was calculated using equation (1).

Inhibition of α -amylase assay

In vitro α -amylase inhibition test was performed based on the method previously described [11]. Each sample determination was carried out in triplicate. The intensity of colour was measured at 540 nm. A graph of percentage inhibitions against sample concentrations was used to obtain the IC_{50} value. α -Amylase inhibitory activity (B), a measure of inhibition, was computed as in Eq 2.

$$B (\%) = \{(1-A)/A_0\}100 \dots\dots\dots (2)$$

where A is the sample's absorbance and A_0 is the control's absorbance.

Inhibition of α -glucosidase assay

Using the procedure described by Zheng *et al* [11], the effectiveness of extracts ability to inhibit α -glucosidase was assessed. The solution's absorbance was measured using a UV-visible spectrophotometer at 400 nm. α -Glucosidase activity (G) was calculated as in Eq 3.

$$G (\%) = \{(A_c - A_s)/A_c\}100 \dots\dots\dots (3)$$

where A_s and A_c are sample and control absorbances, respectively.

Statistical analysis

The concentrations of the tested samples that inhibited 50 % of the enzyme activity (IC_{50}) was calculated using concentration versus inhibitory activity plots, while all the samples were evaluated in triplicates and results are reported as mean \pm standard deviation (SD) using Statistical Software Package for the Social Sciences (SPSS) for computation. Analysis of variance (ANOVA) and least significant differences (LSD) were performed and P -value \leq 0.05 was considered significant.

RESULTS

Extraction yields and phenolic contents

It is evident from the data shown in Table 1 that the yield of aqueous extract varied depending on the harvest region.

The results showed that the reported values are significantly different among the plant extracts of three regions. However, there was no significant difference ($p > 0.05$) in the amount of output as given in Table 1. Aqueous extract of the Al-Tarf regions showed higher total flavonoid content as compared to El-Oued and Tebessa regions.

The yield of aqueous extract of *Mentha spicata* from the El-Oued region (13.4 %) was greater than other two extracts. El-Oued is a Saharan region characterized by high temperature, low humidity and wind. Thus, the plant is subjected to water stress which causes resistance of the plant to climatic conditions which is demonstrated by the high concentrations of total phenolic content (108.94 mg GAE/g DE) compared to *Mentha spicata* harvested from Tebessa and El-Tarf. Igoumenidis *et al* [12] reported that the aqueous extract of *Mentha spicata* from Greece contains 125.7 mg GAE/g DE of total phenolic compounds, which is higher than the values obtained in this work.

Table 1: *Mentha spicata* extract yields, total phenolic content, and total flavonoid content (mean \pm SD)

Zone	El-Oued	Tebessa	El-Tarf	Lsd (0.05)	P-value
Extraction yield (%)	13.4 \pm 1.6	12.7 \pm 1.25	11 \pm 2.21	2.44	> 0.05
Total phenolic content (mg GAE/g DE)	108.94 ^a \pm 11.6	35.89 ^c \pm 2.08	72.88 ^b \pm 5.58	10.64	< 0.05
Total flavonoid content (mg QE/g DE)	0.019 ^b \pm 0.002	0.021 ^b \pm 0.004	0.039 ^a \pm 0.004	0.005	< 0.05

Note: Mean values on the same line followed by similar lowercase letters are significantly different at $p \leq 0.05$

It noted that all the extracts exhibited low flavonoid contents demonstrating a maximum concentration of 0.039 mg QE/g DE for the extract of *Mentha spicata* from the El-Tarf region.

DPPH free radical scavenging activity

The antioxidant potential was evaluated by comparing the absorption of the DPPH solution with the extracts at 515 nm. This variation is due to the hydrogen supply of the antioxidants in the extract which is demonstrated in the form of DPPH-H [10]. The results of free radical scavenging activity determined using DPPH tests are shown in Figure 1. The differences in the percentage inhibition of *Mentha spicata* extracts values are due to changes in region where the plants were cultivated. The highest activity was obtained from extract obtained from plants harvested from the El Oued region. Extracts at 400 $\mu\text{g/mL}$ concentration exhibited 77, 71 and 67 % DPPH scavenging activity from plants harvested at El-Oued, Tebessa and El-Tarf, respectively. Ascorbic acid (used as the control) had DPPH scavenging potency of 100 % at 200 $\mu\text{g/mL}$. The IC_{50} of extracts from plants from El-Oued, Tebessa and El-Tarf were 102.5 ± 2.24 , 223 ± 4.11 and 157.5 ± 2.61 $\mu\text{g/mL}$, respectively.

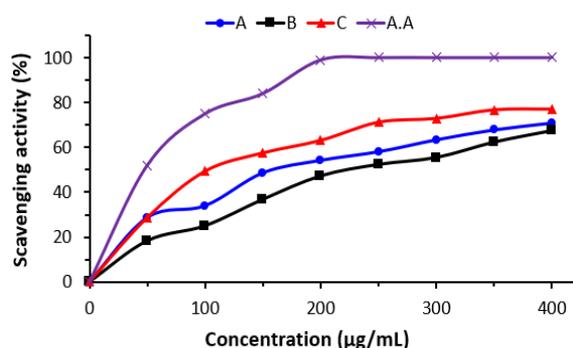


Figure 1: Radical DPPH scavenging activity (%) of the aqueous extracts of *Mentha spicata* obtained from the three regions of Algeria. (A) Tebessa, (B) El-Tarf, (C) El-Oued, (A.A) ascorbic acid

ABTS radical scavenging activity

The aqueous extracts of the leaves of *Mentha spicata* were tested as antioxidants using ABTS assay and the relative antioxidant ability as compared to Vitamin C (ascorbic acid) used as standard. The scavenging activities of extracts shown in Figure 2. The extract of *Mentha spicata* from El-Tarf presents the highest antioxidant capacity with IC_{50} of 111 ± 2.8 $\mu\text{g/mL}$. While the activity of ascorbic acid showed IC_{50} of 56.2 ± 2 $\mu\text{g/mL}$ and it was more potent than obtained for extracts used in this study.

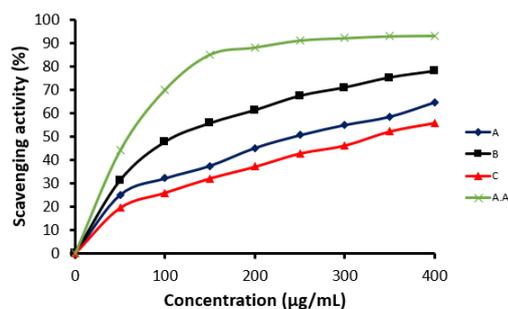


Figure 2: ABTS Scavenging Activity (%) of the aqueous extracts of *Mentha spicata* growing in the three regions of Algeria (A: Tebessa, B: El-Tarf, C: El-Oued, A.A: ascorbic acid)

Ferric-reducing power (FRAP)

Figure 3 shows that all extracts have the ability to reduce Fe^{+3} to Fe^{+2} as absorbances of the extracts is increasing by increasing the concentration, while absorbance for the extracts were taken at 700 nm. For a concentration of 400 $\mu\text{g/mL}$, the absorbance measured was 0.211, 0.391 and 0.691, with extracts of *Mentha spicata* from Tebessa, El-Tarf and El-Oued respectively. Thus, in the concentration range studied only the extract from El-Oued plants exhibited an effective concentration (EC_{50}) of 289.5 $\mu\text{g/mL}$.

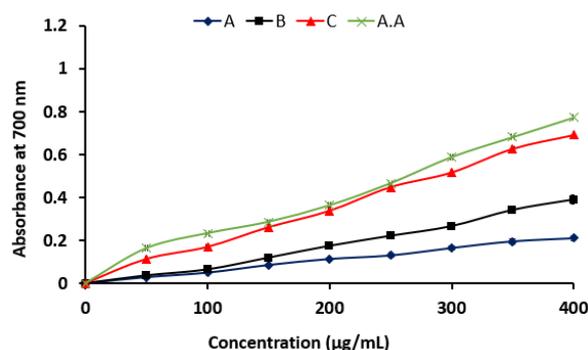


Figure 3: Ferric-reducing power of the aqueous extracts of *Mentha spicata* growing in the three regions of Algeria (A: Tebessa, B: El-Tarf, C: El-Oued, A.A: ascorbic acid)

α -Amylase and α -glucosidase inhibitory activity

The digestive tract converts dietary carbohydrates like starch into simple monosaccharides due to the activity of the enzymes; α -amylase and α -glucosidase. Therefore, blocking the enzymes α -amylase and α -glucosidase can inhibit the digestion of carbohydrates, delay glucose absorption, and subsequently lower blood sugar levels [13]. This plays a crucial role in the treatment of type 2 diabetes [14]. Figure 4 a and b showed that the leaf extracts of *Mentha spicata* from three

different regions have high effectiveness towards both α -amylase and α -glucosidase enzymes. The leaves extracts of *Mentha spicata* from El-Tarf, Tebessa and El-Oued, at a concentration of 400 $\mu\text{g/mL}$ exhibited 56.3, 65.5 and 71.5 % of α -glucosidase inhibition, and 69.6, 67.7 and 71.2 % inhibition of α -amylase inhibition, respectively.

The IC_{50} values of each extract against α -glucosidase and α -amylase are shown in Table 2. Results showed that all extracts exhibited dose-dependent inhibitory activities. The extract obtained from the leaves of *Mentha spicata* from El-Oued exhibited the strongest inhibitory activities for α -amylase and α -glucosidase with IC_{50} as 121.4 and 216.9 $\mu\text{g/mL}$, respectively. There were significant differences ($p \leq 0.05$) between the three extracts of the leaves. Table 2 showed that these extracts exhibit higher ability to inhibit the activity of both α -amylase and α -glucosidase activity. Furthermore, extract from El-Tarf region showed the highest inhibition of α -glucosidase activity than Tebessa extract while, less than El-Oued extract. However, Tebessa extract exhibited significantly highest ability to inhibit the α -amylase activity that El-tarf extracts, while lower than El-Oued extract.

Table 2: IC_{50} values of *Mentha spicata* extracts against α -glucosidase and α -amylase

Source:	IC_{50} ($\mu\text{g/mL}$)	
	α -Glucosidase	α -Amylase
<i>Mentha spicata</i> extract		
Tebessa	251.0 ^b ±9.16	221.2 ^a ±11.36
El-Tarf	291.4 ^a ±8	189.2 ^b ±13.84
El-Oued	216.9 ^c ±7.76	121.4 ^c ±16.4
Acarbose	58 ^d ±1.26	72 ^d ±6.26
LSD (0.05)	11.79	19.2
P-value	<0.05	<0.05

Mean values on the same column followed by similar lowercase letters are significantly different at $p \leq 0.05$

This activity seems to be in line of the highest total phenolic content in this extract which offers a diversity of phenolic compounds that may strongly influence its ability to bind to α -amylase and α -glucosidase enzymes along with their stability and solubility [15]. In fact, the inhibition of α -amylase and α -glucosidase result via binding interactions between phenolic compounds and the catalytic sites of the α -amylase and α -glucosidase. These interactions resulting from hydrogen bonding and hydrophobic interactions [11], which results in π - π conjugation between aromatic ring of α -amylase (or α -glucosidase) and phenolic compounds and induce an incensement of α -amylase (or α -glucosidase) inhibitory activity [16]. However, in comparison to the usual standard drug acarbose, the results were still

lower ($\text{IC}_{50} = 72$ and $58 \mu\text{g/mL}$ for inhibitory activities for α -amylase and α -glucosidase, respectively).

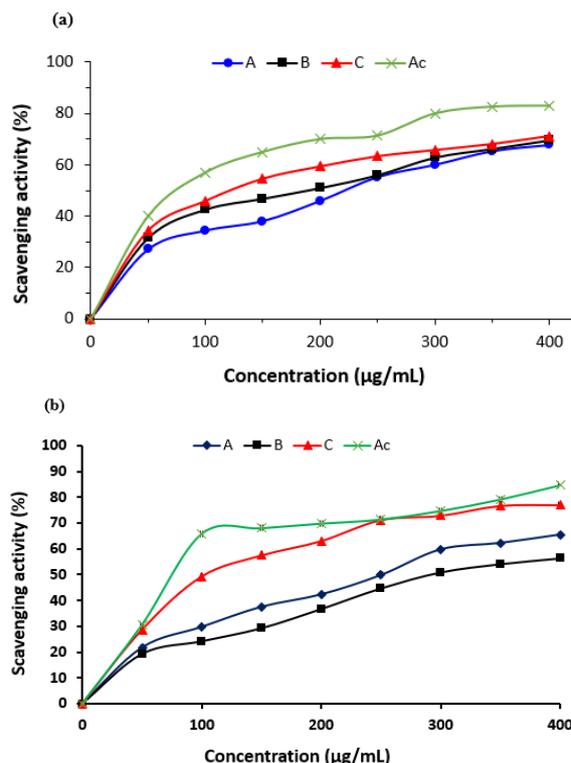


Figure 4: α -Amylase (a) and α -Glucosidase (b) inhibitory activities of the aqueous extracts of *Mentha spicata* growing in the three regions of Algeria (A: Tebessa, B: El-Tarf, C: El-Oued Ac: Acarbose)

DISCUSSION

The growth conditions of a plant (climate and soil) have a significant impact on the nature of the extracts, e.g., the antioxidant capacity and the effectiveness against the action of α -amylase and α -glucosidase enzymes [17]. Considering the effect of the growing medium (temperature, humidity variations, precipitation, and wind) as a determining factor for the qualitative and quantitative estimation of secondary metabolites. Several studies have confirmed that changes in the physical and chemical properties of soil, as well as climatic changes, have a significant impact on the chemical composition of plant extracts, which is consistent with this study [5].

Indeed, the growth conditions determine the characteristics of vegetative growth, the stage of maturity, and therefore the composition of the cell content. The differences in climatic factors and soil characteristics for the three studied areas affected the growth and productivity of the plants and consequently affected their biological activities [5-7].

The altitude of the site can also be a factor. Changes in temperature, sunshine and pressure, affect the plant organ which manufactures the secondary compounds and therefore the capacity for biological activities.

The results showed high activity against the free radical inhibitors of DPPH and FRAP and activity for both α -amylase and α -glucosidase enzymes for the extract from El-Oued region, despite the low yield of the extract. The El-Oued region is characterized by high temperature, wide thermal range, low humidity, scarcity of rainfall and low height above sea level, and sandy soil with high calcium content. This forces the plant to synthesize suitable secondary metabolites, as a suitable defense mechanism adopted by the plant to cope with the harsh environmental and climatic stress [6,17,18]. For the ABTS^{•+} test, the MS of the El-Oued region had a lower IC₅₀ value. This could be because the chemicals included in the extract have different redox potentials, reaction stoichiometry, or both [7].

CONCLUSION

A comparison of chemical profiles, antioxidant activity and the *in vitro* anti-diabetic effects of the aqueous extracts of the leaves of *Mentha spicata* collected from three regions of Algeria (El-Oued, El-Tarf, Tebessa) has been undertaken. The extract of *Mentha spicata* from El-Oued region possesses a significant level of total phenolic contents and antioxidant potential. In effect, soil and climatic factors have a significant impact on the composition of bioactive compounds extracted. All *Mentha spicata* extracts are highly effective at inhibiting the activity of α -amylase and α -glucosidase enzymes as well as possess a high antioxidant potential that are linked with various bioactive constituents which can be further investigated for possible medicinal applications.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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