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Research Article

Phytochemical screening and in vitro antioxidant activities of *Mentha suaveolens* Ehrh. extract

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Abstract: Within the framework of the valorization of the medicinal and aromatic plants of Morocco, we were interested during this study in the characterization and the phytochemical identification of some secondary metabolites present in *Mentha suaveolens* Ehrh. and the evaluation of the antioxidant activity of this species using four methods: DPPH free radical scavenging test, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid, (ABTS) radical cation scavenging test, FRAP test measuring antioxidant power, and β bleaching-carotene. *Mentha suaveolens* is a species belonging to the Lamiaceae family, harvested in southern Morocco, and widely used in traditional medicine for its biological properties attributed mainly to phenolic compounds. Just as they protect plants, secondary metabolites also have a protective role in the human body and are therefore beneficial to our health. They are attributed, in particular, with antioxidant, anti-inflammatory, and antibacterial properties. In this regard, characterization and identification tests of secondary metabolites revealed the presence of alkaloids, flavonoids, catechic tannins, and terpenes in this plant, whereas, the aerial parts of this species are devoid of coumarins, cyanogenic compounds, saponins and free quinones. The quantification of the phenolic compounds gave high contents, with contents of total phenols (54.75 ± 5.62) µg GAE/mg, total flavonoids (32.41 ± 0.41) µg QE/mg, and total condensed tannins (27 ± 1) µg CE/mg. In addition, the results obtained show that *Mentha suaveolens* extract has stronger antioxidant activity using the β-Carotene method with IC50 (0.24 ± 0.06) mg/mL against IC50 (0.021 ± 0.001) mg/mL for the standard antioxidant by the DPPH free radical scavenging test.

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1. INTRODUCTION

Aromatic plants currently present a reliable source of active ingredients known for their therapeutic properties, in particular, antioxidant activity (Saber *et al*., 2021). In this context, a study recently published under the theme, of ethnobotanical, phytochemical, and antioxidant study of fifty medicinal and aromatic plants, the results obtained can be considered as a source of information for scientific research in the field of pharmacology and phytochemistry. With a

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view to finding new bioactive compounds (Afrokh *et al*., 2023). In addition, the secondary metabolites from plants have a natural antioxidant power protecting the human body from free radicals, preventing oxidative stress and associated diseases. For these reasons, they play a very important role in health care (Yao *et al*., 2004). Medicinal plants, therefore, constitute a precious heritage for humanity and can be used in several fields in addition to the therapeutic field, namely the fields of food, cosmetics, perfumery, etc.

In Morocco, aromatic and medicinal plants (AMPs) as natural resources have been a focus of interest in recent years for the national economy. It is one of the richest countries in the world in terms of its diversity: 4200 species of plants, 800 of which are endemic, of which 382 species are known for their medicinal and/or aromatic use (Hmamouchi, 1999; Jamila & Mostafa, 2014). The genus Mentha is one of the important elements of the family Lamiaceae; it is represented by 19 species and 13 natural hybrids (El-Kashoury *et al.*, 2015). Among its species, include *Mentha suaveolens*, which is located in North Africa, Europe, America, and Japan (Sutour *et al.*, 2010).

Mentha suaveolens (MS) or the round-leaved mint has long been known as *M. rotundifolia* (L.) Huds (Harley & Brighton, 1977), is an herbaceous perennial, with a characteristic minty smell, pubescent with a quadrangular stem, with oval leaves, whitish green, covered with network wrinkles. The inflorescences are slender, elongated spikes of small white or slightly pinkish flowers (J. Bellakhdar, 2006). Its flowering takes place from July to September (Kumar, Mishra, Malik, & Satya, 2011). This plant has a wide range of benefits: antispasmodic, analgesic, anti-inflammatory, antimicrobial, acetylcholinesterase, choleretic, carminative, tonic, hypotensive, sedative, stomachic, insecticidal, hepatoprotective, monoamineoxidase inhibitor, it is also applied in the treatment of digestive problems, influenza, respiratory diseases, rheumatism, irritation, skin diseases, nausea, bronchitis and anorexia (Bellakhdar, 1996; Božović, Pirolli, & Ragno, 2015; Karousou *et al.*, 2007; Moreno *et al.*, 2002). In folk medicine, Suaveolens mint products have been used as a carminative (Bellakhdar, 2006). In addition, this species is widely used in the Maghreb to prepare a special pancake that is eaten in winter against the cold. The plant is also used to filter melted butter, which gives it flavor and improves its preservation (Bellakhdar, 2006). The fragrant mint with round leaves contains an essential oil (EO) which can belong to different chemotypes according to the places of harvest. The main components most often encountered are piperitone oxide, piperitone oxide, piperitone, pulegone, neo-isopulegone, carvone, dihydrocarvone (Bellakhdar, 2006).

The main objectives of this study were to characterize the different secondary metabolites present in the aerial parts of MS, which are widely used in southern Morocco for therapeutic purposes, to quantify the levels of phenolic compounds, and to evaluate the antioxidant activity of the methanol extract by following four different methods.

2. MATERIAL and METHODS

2.1. Chemicals and Plant Material

All solvents were of analytical or HPLC grade and purchased from Professional Labo (Casablanca, Morocco). The chemical reagents used in this work are classified as follows: 2,2'-azino-bis (3-ethyl benzthiazoline-6-sulphonic acid), 2,2-diphenyl-1-pycridazil (DPPH 90%), β-Carotene, aluminum chloride (AlCl3), ferric chloride hexahydrate (FeCl3,6H2O), antimony chloride (SbCl3), Iodoplatinate, Dragendorff, Mayer, Neu's reagent, potassium persulphate (K2S2O8), potassium ferricyanide K3Fe(CN)6, Folin-Ciocalteau‘s phenol reagent, sodium carbonate (Na2CO3), sodium hydroxide (NaOH), Gallic acid, Ascorbic acid, Quercetin, Catechin, and Vanillin were sourced from Professional Labo (Casablanca, Morocco).

Mentha suaveolens was collected near the town of Er-rich, located 65km from the province of Errachidia (Latitude: N: 32° 15'33.691", Longitude: O: 4° 29'43.544" and Altitude: 1321 meter) in full bloom during September.

2.2. Phytochemical Screening

With the aid of qualitative characterization reactions, phytochemical screening assays look for various families of secondary metabolites that are present in this plant's aerial portion. These processes rely on precipitating or staining events and use reagents specific to each chemical family (Hagerman AE, 2000). Our study focused on the following compounds: alkaloids, coumarins, cyanogenic compounds, flavonoids, tannins, terpenes, saponins, and quinones.

2.2.1. *Saponosides*

In a beaker, 100 mL of distilled water is added to a quantity of 1 g of dry plant material, then the solution is boiled for 30 min. After cooling, the solution is filtered, and the filtrate is adjusted to 100 mL with distilled water. A series of 1 to 10 mL of filtrate is placed in 10 test tubes, the final volume being readjusted to 10 mL with distilled water. A violent and horizontal agitation was made for 15 seconds for each tube. After 15 minutes of rest, the height of residual foam is measured (in cm) in each tube and whether it is close to 1 cm in the X^{th} tube. The presence of saponins is indicated by a foam index greater than 100. The latter is calculated according to the following relationship (Alilou et al., 2014).

$$I = \text{The height of the foam in the } X^{th} \text{ tube} \times 10/0.0X$$

2.2.2. *Tannins*

A quantity of 1.5 g of dry plant material was placed in 10 mL of 80% methanol and stirred for 15 minutes then filtered on filter paper. A few drops of 1% $FeCl_3$ are added to the methanolic extract already prepared. In the presence of gallic and ellagic tannins, a blue-black coloring is observed, whereas in the presence of catechin tannins, this coloring is greenish brown (Alilou et al., 2014).

2.2.3. *Free quinones*

A quantity of 0.5 g of the dry plant material is placed in 5 mL of petroleum ether. After a few minutes of stirring, the mixture is left to stand for the whole day. After filtering this mixture, it is concentrated using a rotavapor. The change in color of the aqueous phase to yellow, red, or purple after adding a few drops of NaOH (1/10), testifies to the presence of quinones (Alilou *et al*., 2014).

2.2.4. *Terpenoids*

To a quantity of 1 g of the crushed plant material, 5 mL of hexane was added, followed by sonication for 15 minutes. After stirring for 30 min and filtration, migration of the filtrate was carried out on a preparatory silica gel plate, the solvent used is benzene. After migration, the plate is sprayed with antimony chloride (prepared in chloroform) and then placed in an oven at 110° C. for 10 min. Any fluorescence at 365 nm indicates the presence of terpenoids (Alilou *et al*., 2014).

2.2.5. *Coumarins*

Coumarins are detected by two different tests:

*The first test: Detection test A quantity of 2 g of crushed dry plant material is placed in 10 mL of chloroform. Everything is heated for a few minutes and then filtered using filter paper. The migration of this solution was made on a thin layer in the solvent: toluene/ethyl acetate (93/7). After drying under a ventilated hood, the revelation was made using NH_3 vapor under UV at 365 nm (Alilou *et al.*, 2014).

*The second test: Confirmation test 1 g of crushed dry plant material is weighed and placed in a test tube, in the presence of a few drops of water, the tube is covered with filter paper soaked in diluted NaOH. The whole is placed in a boiling water bath for a few minutes. The filter paper is then examined under UV light at 365nm. Any yellow fluorescence indicates the presence of coumarins (Alilou et al., 2014).

2.2.6. *Cyanogenic compounds*

A quantity of 1g of fresh plant material is wetted with a few drops of chloroform $CHCl_3$ in a test tube where a strip of filter paper impregnated with sodium picrate is inserted. The whole is heated in a water bath at 35°C. for 3 hours. A red turn of the strip after the production of HCN indicates the presence of cyanogenic compounds (Alilou *et al*., 2014).

2.2.7. *Alkaloids*

The presence of alkaloids has been demonstrated by three different tests, which have a qualitative purpose: The Iodoplatinate, Dragendorff, and Mayer tests (Alilou *et al*., 2014), because some alkaloids may be sensitive to certain tests and not detectable by others.

*Preparation of methanolic extracts: Two grams of plant material, dry and ground, is added to 100 mL of 80% methanol. After sonication for 15 min and stirring overnight, the extracts are filtered and evaporated to dryness using a rotary evaporator. The residues are taken up in a few ml of pure methanol. These extracts are subjected to the following two tests:

*Iodoplatinate test: The methanolic extract to be tested is deposited on a thin layer (silica plate) the chromatogram is developed in the following solvent: (AcEt/MeOH/NH_4OH) (9/1/1), then dried under a fume hood. The migration bands are identified and delimited under UV light at 365 nm. Application of the Iodoplatinate reagent by spraying reveals the presence of alkaloids. These show up as a blue to purple color on the chromatogram.

*Dragendorff test: It is based on the same principle as the Iodoplatinate test, except that it is revealed by spraying with Dragendorff reagent. The appearance of bright orange spots on the chromatogram indicates the presence of alkaloids.

*Mayer test: To a quantity of 0.5 g of crushed dry plant material, 15 mL of ethanol (70%) is added and in order to destroy the cell walls and release all the constituents that bathe in the vacuole, sonication is carried out for 15 min. Then, the extracts are left under magnetic stirring overnight. After complete decantation, filter through filter paper. The extract is evaporated to dryness in the rotavapor. The residue recovered in a few ml of HCl (50%) is then transferred to two test tubes; one is used as a control and to the other Mayer's reagent is added. The appearance of a white precipitate reflects the presence of alkaloids.

2.2.8. *Flavonoids*

One gram of dry powder plant material is extracted with 20 mL of 80% MeOH. After stirring for 15 min and another 15 min of sonication, the extracts are filtered and subjected to TLC, the migration solvent being glacial acetic acid/H_2O (15/85). Visualization is done at 365 nm after spraying with Neu's reagent (2-aminoethyl diphenyl borate) at 1% in pure MeOH. (Dohou *et al.*, 2003).

2.3. Quantitative Determination Assays

2.3.1. *Total phenolic content*

The quantification of the phenolic content of the methanol extract was determined using the Folin- Ciocalteu method (Singleton *et al.*, 1999). Briefly, 100 µL of extract or standard antioxidant (gallic acid (GA)) was mixed with 500 µL of the Folin-Ciocalteu reagent (10 times diluted in distilled water). After 2 minutes of incubation, 2.00 mL of the 20% Na_2CO_3 solution was added. The mixture was left to settle for 30 minutes at room temperature in the dark, and

the absorbance is read at 765 nm using LLG-uniSPEC 2 Ultraviolet–Visible spectrophotometer, against a blank without extract. The produced solutions' optical densities were utilized to draw the GA calibration curve. Total phenolic content was calculated from the linear regression equation (**y** = 0,004x + 0,089 with a correlation coefficient R^2 = 0.982) of gallic acid and expressed as micrograms of gallic acid equivalent per one milligram of extract (µg GAE/mg of extract). All measurements are performed in triplicate.

2.3.2. *Total flavonoids content*

The total flavonoid content of the extract was determined by the colorimetric method using aluminum trichloride as described by (Quettier-Deleu *et al*., 2000). Briefly, 1.00 mL of plant extract or quercetin standard solution was mixed separately with 1.00 mL of 2% aluminum chloride. After 10 minutes in the dark and at room temperature, the absorbance is read at 430 nm with a spectrophotometer (LLG-uni spectrophotometer). The total flavonoid concentration is calculated from the regression equation of the calibration range established with standard quercetin prepared in methanol (y=0.006x+0.079 with a correlation coefficient R^2=0.991). The result is expressed in micrograms of quercetin equivalent per gram of extract (µg QE/mg of extract). All tests are repeated three times.

2.3.3. *Total condensed tannin content*

The quantification of condensed tannins (proanthocyanidins) in the extract using the method of Sun *et al.* in 1998 (Sun, Ricardo-da-Silva, & Spranger, 1998). 50 µL of the sample or diluted standard was mixed with 3 mL of a 4% vanillin-methanol solution and 1.5 mL of 37% hydrochloric acid was added. 15 minutes were given for the mixture to stand. at room temperature. The absorbance was then measured at 500 nm against a water/methanol mixture (v/v) as a blank. A calibration curve was produced in parallel under the same operating conditions using catechin (C). The total condensed tannin contents are calculated from the regression equation of the calibration range established with catechin (y=0.001+0.025, R^2=0.996, where y was the absorbance and x was the concentration). The result is expressed in micrograms of catechin equivalent per gram of extract (µg CE/mg of extract). All tests are replicated three times.

2.4. Evaluation of Antioxidant Activity

2.4.1. *DPPH free radical scavenging activity*

The free radical scavenging capacity of the methanolic extract was determined using the stable free radical, 2,2 diphenyl-1-picryhydrazyl (DPPH•) according to the method explained by (Loo, Jain, & Darah, 2008). Briefly, 1.80 mL of a 0.004% methanol DPPH solution is added to 0.20 mL of various concentrations of methanol extract or standard and allowed to stand in the dark for 30 minutes for the reaction to occur. The absorbance of the mixture is measured at 517 nm using a spectrophotometer (LLG-uni spec2spectrophotometer). The IC50 value (50% inhibitory concentration) was calculated and obtained from the linear regression (Molyneux, 2004). A low IC_{50} value indicates high antioxidant activity. The experiment was done in triplicate.

2.4.2. *Ferric reducing antioxidant power (FRAP)*

The reducing power of iron (Fe^{3+}) in the extract is determined according to the method described by Oyaizu (Oyaizu, 1986). One milliliter of the extract at different concentrations is mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH= 6.6) and 2.5 mL of a solution of $K_3Fe(CN)_6$ at 1%. The whole is incubated in a water bath at 50°C for 20 min, then 10% trichloroacetic acid (2.5 mL) is added to stop the reaction, and the tubes are centrifuged for 10 min at 3000 rpm. An aliquot (2.5 mL) of supernatant is combined with 2.5 mL of distilled water and 0.5 mL of an aqueous solution of ($FeCl_3$, $6H_2O$) at 0.1%. The reading of the absorbance of the reaction medium is done at 700 nm against a similarly prepared blank, replacing the extract

with distilled water which makes it possible to calibrate the device (LLG-uni spec2spectrophotometer). The positive control is represented by a solution of a standard antioxidant whose absorbance was measured under the same conditions as the samples.

2.4.3. *ABTS radical cation scavenging assay*

The solution of ABTS radical cation **(**$ABTS^{\bullet+}$) is prepared by mixing 2 mM of an ABTS with 70 mM of a solution of potassium persulfate (v/v). Before usage, the mixture is stirred for 24 hours in the dark and at room temperature. The solution was then diluted with methanol to achieve an absorbance of 0.700±0.02 at 734 nm. 2 mL of this solution, 200 µL of extract or positive control are added, and the absorbance obtained after 30 min at 734 nm is noted (Müller, Fröhlich, & Böhm, 2011).

2.4.4. *β-Carotene bleaching test*

The antioxidant activity of the aqueous solution was determined by a β-carotene/linoleic acid system (Bougatef *et al.*, 2009). Briefly, in a round bottom flask, we put 40 µL of linoleic acid, 1 mL of β-carotene solution (2 mg/mL in chloroform), and 400 µL of Tween 20. A stream of nitrogen was used to evaporate the chloroform from the mixture. Then, distilled water (100 mL) was slowly added to the residue and vigorously stirred to give a stable emulsion. A 2.5 mL aliquot of this emulsion was added to 500 µL of methanolic solution of MS prepared at different concentrations. 500 µL of distilled water was added to the control reaction mixtures. At 470 nm, absorbance was immediately measured. After 120 minutes, the absorbance of the tubes was measured in a water bath at 50°C.

3. RESULTS and DISCUSSION

3.1. Phytochemical Screening

The results of the screening tests carried out on the aerial parts of MS, are shown in Table 1. The characterization tests made it possible to identify the main chemical groups contained in the aerial parts of MS such as catechin tannins, flavonoids, terpenes, and alkaloids. On the other hand, the other families such as free quinones, coumarins, saponosides, cyanogenic compounds, and Gallic tannins were not detected (Figure 1).

Figure 1. Phytochemical screening of *Mentha suaveolens*

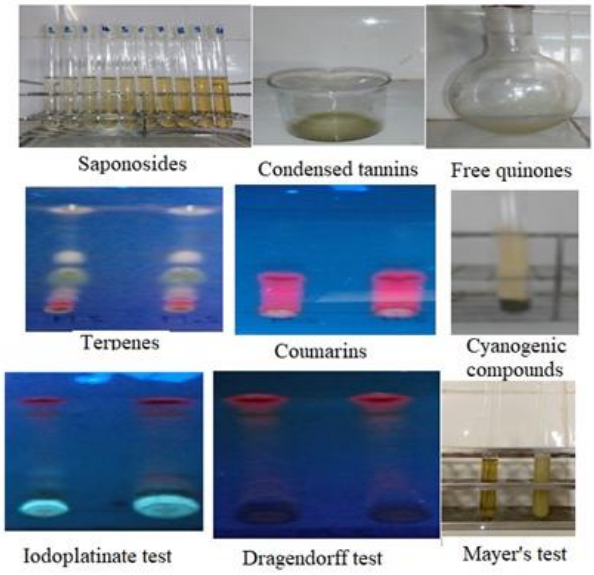

Table1. Results of phytochemical screening of aerial parts of *Mentha suaveolens*

	Secondary metabolites		Solvent	Reagent/developer Without or with UV 365nm	Observations	Results
Mentha suaveolens	Saponosides		Eau distillée	Foam indices	Foam height 0.6	-
	Condensed tannins	Catechin	Methanol	Iron chloride ($FeCl_3$) à 1%	Greenish brown	++
		Gallic			-	-
	Free quinones		Petroleum ether	NaOH 0,1 N	-	-
	Terpenes		Benzene	Antimony chloride	Sky blue	+++
	Coumarins		Toluene/ethyl acetate: (93/7)	NH_3 vapors	-	-
	Cyanogenic compounds		Chloroform	Sodium Picrate	Yellow	-
	Alkaloids		AcEt/MeOH / NH_4OH : (9/1/1)	Dragendorff	Orange to brown	++
				Iodoplatinate	Blue to purple	
			Methanol	Mayer	Yellowish white	
	Flavonoids		Glacial acetic acid/water: 15/85	Neu reagent	Light blue, Yellow and Dull yellow	++

+++ : Strongly test
++ : Positive average test
+ : Low positive test
- : Negative test

The effective presence of certain secondary metabolites and the absence of others does not exclude the therapeutic properties of these plants (Kabran, 2011). These compounds are known for their bioactive properties. Flavonoids are very effective and non-toxic antioxidants; they are antispasmodic, anti-ulcer, anti-secretory, anti-allergic, anti-diarrheal, and anti-inflammatory, and they protect against cancer and cataracts (Bimakr *et al*., 2011; Bruneton, 2009). There are pharmacological activities associated with alkaloids such as enhancement of cardiac activity, excitation of the central nervous system and symptomatic nerves, and stimulation of blood circulation (Kabran, 2011). Certain diseases may also be treated with these plants due to their alkaloids (N'Guessan *et al*., 2009). Tannins show the properties of vitamin D, they could be utilized to support blood vessels and aid in the body's absorption of vitamin C. (Kabran, 2011). As for terpenes, they are used as additives in the food and cosmetic industries (Tsao & Coats, 1995), and numerous of them have biological activities: anti-carcinogenic, antimicrobial, anti-inflammatory, insecticidal (Murakami *et al*., 2004), anesthetic and antihistamine (mono and sesquiterpenes), diuretic (β-eudesmol) *(Veličković et al*., 2003; Hsiou, 2000), neuroprotective (α-terpinene, γ-terpinene, and trans-caryophyllene) (Chang, Kim, & Chun, 2007). Anti-tumor and cytotoxic properties of diterpenes (taxol) and the antioxidant activities attributed above all to phenolic diterpenes (Gill, 1993) may also be mentioned. The presence of coumarins explains the antifungal (Kandaswamy & Raveendiran, 2014), antibacterial (Bhat, Al-Omar, & Siddiqui, 2013), antiviral (R. W. Fuller, 1994)), antimalarial (Yang *et al*., 1992), anti-inflammatory (Bhat *et al*., 2013; Chang *et al*., 2007; García-Argáez *et al*., 2000; Gill, 1993; Hiermann, Schramm, & Laufer, 1998; Kandaswamy & Raveendiran, 2014; Milcent & Chau, 2003; Murakami *et al*., 2004; Fuller, 1994); Tsao & Coats, 1995; Veličković *et al*., 2003; Hsiou, 2000; Yang *et al*., 1992), anti-tumor (Fujioka *et al*., 1999; Kofinas *et al*., 1998) and anticoagulant effect (Egan, 1990).

3.2. Estimation of The Phenolic Compound Content

The results of the assay of the phenolic compounds in the methanolic extract of the aerial parts of MS are grouped in Table 2.

Table 2. Contents of phenolic compounds in the aerial parts of *Mentha suaveolens.*

The phenolic compounds	Total phenolic (µg GAE /mg)	Total flavonoids (µg QE/mg)	Total condensed tannin (µg CE/mg)
Content	54.75 ± 5.62	32.41 ± 0.41	27 ± 1

According to our results cited in the table above, it appears that the content of total phenols in our methanolic extract of MS (54.75 ± 5.62) µg GAE /mg of extract) is lower than those of Salhi et al. 2017(145±7.48) µg GAE /mg (Salhi et al., 2017), whereas, the content of total flavonoids in our methanolic extract of MS (32.41 ± 0.41) µg QE/mg of extract) is closer to those of Salhi *et al*. (2017) (30.57±2.13) µg QE/mg(Salhi *et al*., 2017) and also higher than those of Bichra *et al*. (2013) (0.1 ± 0.02) CE µg /mg and (0.3 ± 0.08) CE µg /mg Dry Matter (DM) for the aqueous and phenolic extract, respectively (Bichra, El-Modafar, El-Abbassi, Bouamama, & Benkhalti, 2013). Additionally, the condensed tannin content for our methanolic MS extract is (27 ± 1) µg CE/mg extract.

3.3. Evaluation of Antioxidant Activity

In accordance with the results gathered in Table 3, it is important to emphasize that the methanolic extract of *Mentha suaveolens* has a slightly moderate antioxidant power for the four tests at the rate of IC50: (0.29 ± 0.02) mg/mL, (0. 25 ± 0.03) mg/mL and (0.28 ± 0.01) mg/mL, (0.24 ± 0.06) mg/mL for the tests: DPPH$^{\bullet}$ Free radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP), ABTS$^{\bullet+}$ Radical Scavenging Test and Whitening of the β-

Carotene, respectively but remains less important than the standard antioxidant, ascorbic acid for the four tests at the rate of IC50: (0.021 ± 0.001) mg/mL, (0.022 ± 0.004) mg/mL, (0.031 ± 0.002) mg/mL, (0.027 ± 0.005) mg/mL, respectively for the same tests mentioned above in the same order. By comparing our results with those of the literature for the DPPH test, it appears that the value obtained for the methanolic extract of MS is in good agreement with those of Madiha *et al*. (2012) (Bichra & Benkhalti, 2012), particularly in terms of inhibition percentages and higher than Kasrati *et al*. (2017) (19.51 ± 0.04 µg/mL), especially for *Mentha suaveolens*.

According to our results for the FRAP test, we can suggest that the reducing power of our extract is probably due to the presence of hydroxyl group in the phenolic compounds which can serve as an electron donor. Therefore, antioxidants are considered reducers and inactivators of oxidants (Siddhuraju & Becker, 2007). To our knowledge, no study has been carried out on the methanolic extract of MS using the $ABTS^{\bullet+}$ cation radical reduction test.

In addition, according to a study conducted by Kasrati *et al*. (2017) (Kasrati *et al*., 2017) on *Mentha suaveolens* subsp timija (Briq.) Harley using the β-Carotene bleaching test, it turns out that their extract (IC_{50}= 64.92 ± 0.90 µg. mL^{-1}) has an antioxidant effect more important than ours.

Table 3. Antioxidant activity of MS *extract* using DPPH, ABTS, FRAP and β-Carotene bleaching methods.

Sample/Standard	Antioxidant activity tests			
	DPPH IC_{50}(mg/mL)	FRAP IC_{50}(mg/mL)	ABTS IC_{50}(mg/mL)	β-Carotene bleaching IC_{50}(mg/mL)
Methanolic extract	0.29 ± 0.02	0.25 ± 0.03	0.28 ± 0.01	0.24 ± 0.06
Ascorbic acid	0.021 ± 0.001	0.022 ± 0.004	0.031 ± 0.002	0.027 ± 0.005

4. CONCLUSION

This work concerns the phytochemical study of a species belonging to one of the major plant families serving as a framework for evaluating the presence of certain typical secondary metabolites. Just as they protect plants, secondary metabolites also have a protective role in the human body and are therefore beneficial to our health. They are attributed in particular with antioxidant, anti-inflammatory, and antibacterial properties. In this regard, characterization and identification tests of secondary metabolites revealed the presence of alkaloids, flavonoids, catechic tannins, and terpenes in this plant. But, the aerial parts of this species are devoid of coumarins, cyanogenic compounds, saponins, and free quinones. The quantification of phenolic compounds gave high contents, with contents of total phenols (54.75 ± 5.62) µg GAE /mg, total flavonoids (32.41 ± 0.41) µg QE/mg, and total condensed tannins (27 ± 1) µg CE/mg. In addition, the results obtained show that the extract of this plant has a stronger antioxidant activity for the β-Carotene method with IC_{50} (0.24 ± 0.06) mg/mL against IC_{50} (0.021 ± 0.001) mg/mL for the standard antioxidant by the test DPPH radical scavenging. However, the results of this study showed that the extract of the aerial parts of this plant has antioxidant capabilities that could act as free radical scavengers or inhibitors or possibly act as a natural antioxidant.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Moha Afrokh: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft**. Kamal Boumhara:** Investigation, Resources**. Khalid Chatoui:** Investigation, Resources, Visualization, Methodology**. Saida Tahrouch and Abdelhakim Hatimi**: Methodology, Supervision, and Validation. **Hicham Harhar** and **Mohamed Tabyaoui**: visualization, editing the original draft.

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