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Exploring of Antifungal and Antibacterial Effects of Green Mint (*Mentha spicataL.*) on Microbial infection

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Abstact *:Plants possess extraordinary therapeutic virtues. They contain a large number of molecules with multiple applications in the pharmaceutical industry, food, cosmetics, and dermopharmacy. Much of current research focuses on the study of antimicrobial molecules, making it interesting to position our work within this research context. The objective of our study is to evaluate the antibacterial and antifungal activity of extracts from the leaves of Mentha spicata from the Saida and El-Bayedh regions against microbial strains.Our extract preparation was carried out through maceration using 80% methanol. The total polyphenol content was determined by spectrophotometry using the Folin-Ciocalteau method, the total flavonoid content was estimated using a colorimetric method with aluminum chloride solution, and the condensed tannins were measured using a vanillin-HCl test.The antibacterial activity was assessed using the aromaticogram method with the well diffusion technique. The extracts of Mentha spicata were tested on two culture media, Muller Hinton and Sabouraud, against four pathogenic strains: Escherichia coli (ATCC25922), Staphylococcus aureus (ATCC 25923), Candida albicans (ATCC 10231), and Bacillus cereus (ATCC 11778).Experimental results revealed that the extract from the Saida region is richer in total polyphenols than the extract from El-Bayedh, with values of 180.16 and 138.5 mg EAG/g of extract, respectively. It also contained more flavonoids (6.15 and 5.1 mg EQ/g of extract) and higher levels of condensed tannins (72 and 57.83 mg EC/g of extract from Saida, being more effective than the extract from El-Bayedh).Regarding antibacterial activity, our results show that S. aureus is sensitive to the concentrations of 20% (IZ = 10 mm, 8 mm for Saida and El-Bayedh, respectively), 40% (IZ = 14 mm, 13 mm), and very sensitive to 60% (IZ = 16 mm, 15 mm), 80% (IZ = 17 mm, 15 mm), and 100% (IZ = 19 mm, 16 mm). Bacillus cereus is resistant at 20%, 40%, 60%, and 80%, but sensitive at 100%, with a diameter of 9 mm for both extracts. E. coli is sensitive at 20% (IZ = 9 mm, 8 mm), 40% (IZ = 10 mm, 10 mm), 60% (IZ = 10 mm, 11 mm), 80% (IZ = 11 mm, 11 mm), and 100% (ZI = 12 mm, 11 mm). Candida albicans is resistant at 20% and sensitive at 40% (IZ = 9 mm, 8 mm), 60% and 80% (ZI = 10 mm, 8 mm), and at 100% (IZ = 14 mm, 12 mm).These findings encourage further studies on extracts from a variety of medicinal plants to maximize their benefits.*

Keywords *:Mentha spicata L., Extract, antibacterial activity, total polyphenols, total flavonoids, condensed tannins, bacterial strains, Saida, El-Bayedh.*

INTRODUCTION

Since the 1980, medicinal plants have made a strong comeback, relying on proven values established by ancestors over the years. Several factors are behind this renewed interest, such as lower costs compared to conventional medications, relative availabilityespecially in remote areasmistrust of synthetic products, and a desire to consume "organic." Today, although we have seen the spectacular development of synthetic drugs, many countries, including developed nations, continue to rely on traditional remedies. The World Health Organization (WHO) reports that between 75% and 95% of rural communities, especially in developing nations, rely on traditional medicine primarily derived from plants (WHO, 2003). Research indicates that around 20% of plant species globally possess therapeutic or cosmetic properties due to the presence of active compounds with diverse biological effects, which are utilized across various sectors such as medicine, pharmacy, cosmetology, and agriculture (Suffredini, 2004).

Antibiotic resistance is a biological phenomenon that medicine will struggle to eliminate. A few decades ago, several diseases seemed to be under control thanks to the use of antibiotics. Scientific and technological advancements even suggested the possible eradication of many pathologies. However, the

increasing resistance developed by microorganisms and the regular emergence of new infectious agents have contradicted this optimistic outlook. For over twenty years, numerous resistance determinants have been described alongside the rise of increasingly resistant bacteria. Algeria is among the Mediterranean countries with the richest phylogenetic resources of aromatic and medicinal plants, thanks to its varied bioclimatic zones. Among the 3,150 plant species found in the country, over 300 are utilized for therapeutic or aromatic purposes (Morales, 2002).

Mints are phyto-aromatherapy plants widely used in perfumery, confectionery, and pharmaceutical preparations.They are also used in various traditional therapeutic treatments as natural remedies, as well as in the food industry as additives or to enhance flavor, due to their olfactory properties. The Lamiaceae family includes about 220 genera and 3,300 species, widely distributed and utilized for various purposes worldwide. Species belonging to the Lamiaceae family are rich in phenolic compounds, many of which are known for their antioxidant, antibacterial, and antifungal properties(Barchan et *al*., 2015 ; Brahmi et *al*., (2016 ; Almeida et *al*., 2012) .

In Algeria, the literature reports the occurrence of six species of the *Mentha* genus: M. *rotundifolia*, M. *spicata*, M. *pulegium*, M. *piperita*, M. *longifolia*, and M. *aquatica*, as well as three hybrids of these species: M. *durandoana*, M. *niliaca*, and M. *schultzii*(Quezel and Santa, 1963). The same reference noted that M. *longifolia* and M. *aquatica* were very rare at that time and are likely decimated now.

In this context, we conducted this study to evaluate the antimicrobial and antifungal activity of methanolic extracts from the aerial parts of green mint (*Mentha spicata* L.) against three pathogenic strains: *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.

MATRIELS AND METHODES

Objective: The evaluation of the antibacterial and antifungal activity of extracts from the leaves of the species *Mentha spicata* from the regions of Saida and El-Bayadh against bacterial strains.

Work Location: This study was conducted in the General Microbiology and Biochemistry laboratory of the Faculty of Natural and Life Sciences at Sidi Bel Abbès University.

Collection Region and Preliminary Treatments of Plant Material: The leaves of *Mentha spicata* studied were collected from two regions in Algeria, namely Saida and El-Bayadh, where the differing climatic, edaphic, and ecological conditions may influence the composition of bioactive compounds in the plants.

3.1 Region of El-Bayadh

3.1.1 Location and Site Characteristics: The El-Bayadh region is located approximately 450 km southwest of the capital (33° 41' 10" N, 1° 0' 50" E) at an altitude of 1340 meters, belonging to the southern High Plains of Oran, which is part of a geographical area known as the Algerian steppe. This specific area is situated between two mountain ranges: the Tell Atlas to the north and the Saharan Atlas to the south (A.N.A.T, 2009; cited in Mammeri, 2009).

The study area is precisely bordered to the north by the Chott Cherrgui depression, to the east by the Ksours mountains, to the south by the western Erg, and to the west by Djebel Amour. It covers an area of 71,693.70 km².

4. Bacterial Strains

Antibacterial tests were performed on common pathogens responsible for various diseases. The microorganisms studied and their references are listed in the following table1:

Table 1: Bacterial strains and their references

Bacteriumor fungus	Reférences	Gram+/-
Bacillus cereus	ATCC 11778	Positive
Staphylococcus aureus	ATCC 25923	Positive
Escherichia coli	ATCC 25922	Negative
Candida albicans	ATCC 10231	/

5 PLANT MATERIAL

The leaves are separated from the stems, and the petioles are removed from the leaves. Sorting is done by discarding any stained or diseased leaves.

5.1 Drying

A sample of 2 kg of plant material, taken exclusively from the aerial part of the studied *Mentha spicata*, was randomly collected from each experimental region. The plant material was then spread out on aluminum foil and dried in ambient air.

The dried leaves were finally ground in a kitchen blade grinder and placed in airtight jars, stored in a dry environment (at room temperature) and protected from moisture.

5.2 Grinding

This operation was performed after drying the collected plants in an electric grinder.

6. METHODS

6.1 Extract Preparations: For each region, 2 g of the powdered dried and ground leaves were subjected to maceration with 50 ml of methanol (80% v/v) for 24 hours at room temperature. The duration of the extraction will thus promote the depolymerization of the main constitutive compounds of the plant, such as lignin and pectic substances, allowing better solubilization of the main bioactive compounds (Messaoud et *al.*, 2011). The extraction of the latter was performed separately for each region using samples of 8 g of powder for 200 ml of methanol (80% v/v) for 24 hours at room temperature.

6.2.Yield Calculation

The yield of the different extracts obtained is defined as the ratio between the mass of the dry extract obtained and the mass of the plant material used. This yield is calculated using the following equation (Carré, 1953):

$R(\%)=(Me/Mv)\times 100$

where:

R(%): Yield in %,

Me: Mass of the extract after solvent evaporation,

Mv: Mass of the plant material used for extraction.

The pure extracts rich in recovered bioactive compounds were finally diluted in DMSO at varying rates of 0, 20, 40, 60, 80, and 100%, respectively.

6.3 Dosage of Phenolic Compounds : In order to determine the phenolic compound content of the extracts from *Mentha spicata* L leaves, three protocols were followed to measure the total phenols, flavonoids, and tannins.

6.3.1 Total Polyphenol Assay : The total polyphenol content is determined by spectrophotometry using the Folin-Ciocalteu method (Ben El Hadj Ali et al., 2014). All these compounds are oxidized by the Folin-Ciocalteu reagent. This reagent, which is orange in color, consists of a mixture of phosphotungstic acid and phosphomolybdic acid that are reduced during the oxidation of phenols to a mixture of blue tungsten and molybdenum oxides. The blue coloration produced is proportional to the concentration of phenolic compounds and has a maximum absorption around 765 nm.

For each assay, 500 µl of appropriately diluted extract is mixed with 2 ml of Folin-Ciocalteu reagent (diluted 10 times in distilled water) and incubated in the dark for 5 minutes. After incubation, 2.5 ml of sodium carbonate (7.5%) is added. The mixture is kept in the dark and incubated for 90 minutes at room temperature. The optical density is read at 760 nm. For each sample, the experiment is repeated three times

The polyphenol contents of the different extracts are expressed as gallic acid equivalents per gram of dry matter (mg EAG/g DM).

6.3.2 Total Flavonoid Assay : The quantity of flavonoids was estimated using a colorimetric method with an aluminum chloride ($AlCl_3$) solution. The assay is based on the formation of a colored complex (yellow) between flavonoids and aluminum chloride, which absorbs at a wavelength of 430 nm. The intensity of the yellow color is proportional to the concentration of flavonoids in the extracts.

To perform this, 500 µl of the diluted extract is mixed with 500 µl of $AlCl_3$ (2% in methanol) and then incubated in the dark for 40 minutes at room temperature. The absorbance is measured at 430 nm (Koolen

et *al.*, 2013).The flavonoid content is expressed as quercetin equivalents per gram of dry matter (mg EQ/g extract).

6.3.3. Condensed Tannin Assay : The quantity of condensed tannins present in the methanolic extracts was determined using a vanillin-HCl test according to the method described by Rebaya Aet al. (2015). This method is based on the reactivity of vanillin in an acidic medium with condensed tannins to form anthocyanidols, which are red chromophores detectable by spectrophotometry.

In this test, 12.5 µl of extract is added to 750 µl of vanillin (4% in methanol) and 375 µl of concentrated HCl. The mixture is blended and incubated for 15 minutes at room temperature, and the absorbance is measured at 500 nm.The results are expressed as milligrams of catechin equivalents per gram of dry matter (mg EC/g DM).

6.3.4. Gel Diffusion Method (Well Diffusion) :The method used is the well diffusion method on agar as described by Berghe and Vlietinck (1991).

-Technique

Mueller Hinton and Sabouraud agar media are poured into Petri dishes to a thickness of 8 mm.Inoculation by swabbing the dilutions of the microorganisms to be tested is carried out according to a Mac Farland scale.Wells with a diameter of 6 mm are made concentrically on the media, and then 0.1 ml of each concentration of the extracts is added to each well at the center.

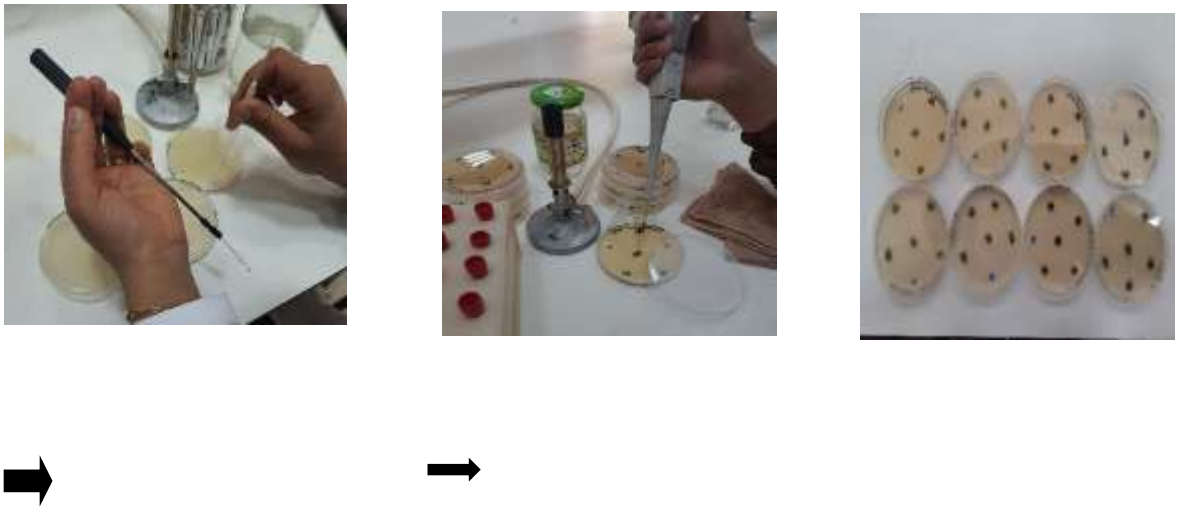

Figure 1: Application of extracts at different concentrations on inoculated plates.

After a pre-diffusion period of 45 minutes at room temperature, the strains are incubated at 37°C for 24 hours for *E. coli*, *S. aureus*, and *Bacillus cereus*, and for 72 hours for *Candida albicans*, after which the diameters of the inhibition zones are measured using a caliper.

RESULTS

Calculate the yield of the species from the Wilaya of Saida.

Calculate the yield of the species from the Wilaya of Elbayadh.

$(2.3/8)*100 = 28.75\%$

$(2.1/8)*100 = 26.25\%$

. Quantification of Phenolic Compounds

3.1 Quantification of Total Polyphenols

After 90 minutes of incubation at room temperature, we observed a color change to dark blue, confirming the richness of the extracts in total polyphenols.

Les teneurs en phénols totaux des extraits des feuilles de *Mentha spicata L.*, exprimées en mg équivalent acide gallique par gramme d'extrait sec, sont représentées dans la figure ci-dessous :

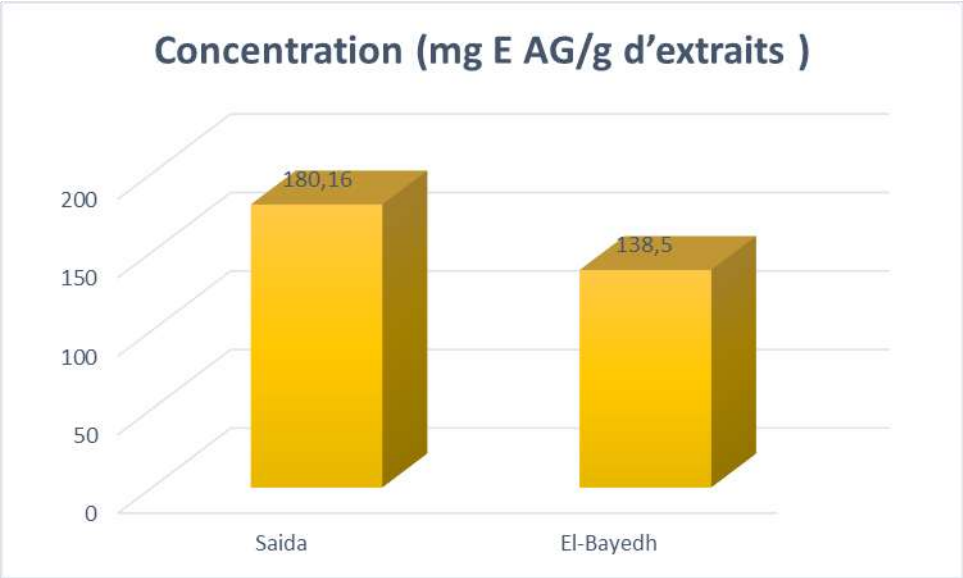

Figure 2: Total Polyphenol Content of Extracts from *Mentha spicata* L. Leaves.

The results reveal that the total polyphenol content of extracts from *Mentha spicata* L. leaves from the Wilaya of Saida is higher compared to that from the Wilaya of El-Bayedh.

3.2. Quantification of Flavonoids

After 45 minutes of incubation at room temperature, a yellow color change was observed in the three tubes for each extract, indicating the presence of flavonoids.

The results of the flavonoid quantification from extracts of *Mentha spicata* L. leaves, expressed in mg of quercetin equivalents per gram of dry extract, are represented in the figure below :

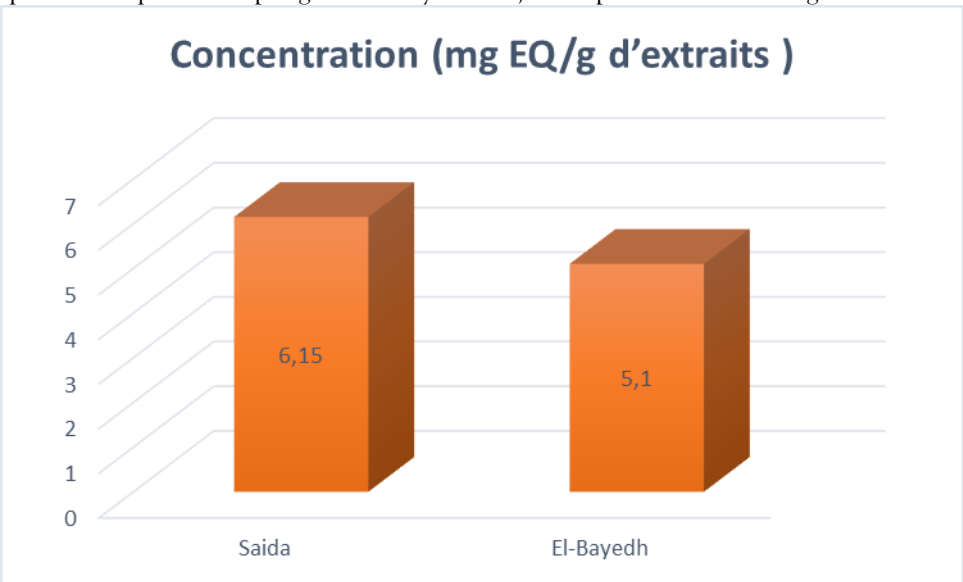

Figure 3: Flavonoid Content of Extracts from *Mentha spicata* L. Leaves

The results show that the flavonoid content of extracts from *Mentha spicata* L. leaves from the Wilaya of Saida is higher compared to that from the Wilaya of El-Bayedh.

3.3 Quantification of Condensed Tannins

A light pink color was observed after 15 minutes of incubation at room temperature.

The contents of condensed tannins in extracts from *Mentha spicata* L. leaves, expressed in mg of catechin equivalents (CE) per gram of dry extract, are represented in the figure below:

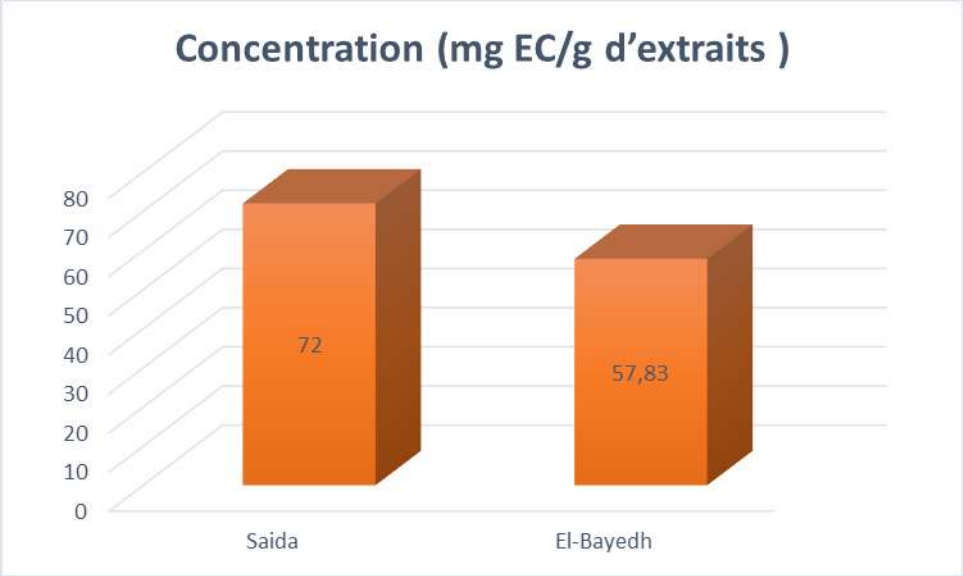

Figure 4: Condensed Tannin Content

The results show that the condensed tannin content of extracts from *Mentha spicata* L. leaves from the Wilaya of Saida is higher compared to that from the Wilaya of El-Bayedh.

Antibacterial Activity of Extracts

The antimicrobial activity of the two methanolic extracts of our plant is tested against four bacterial strains using the agar diffusion method. The antimicrobial power of each extract is estimated in terms of the inhibition diameter represented by a clear halo formed around each well, expressed in mm and interpreted in four levels of activity according to Ponce et *al.* 2003 as follows:

(-) resistant strain: D < 8 mm.
(+) sensitive strain: 9 mm ≤ D ≤ 14 mm.
(++) very sensitive strain: 15 ≤ D ≤ 19 mm.
(+++) extremely sensitive strain: D ≤ 20 mm.

Staphylococcus aureus :

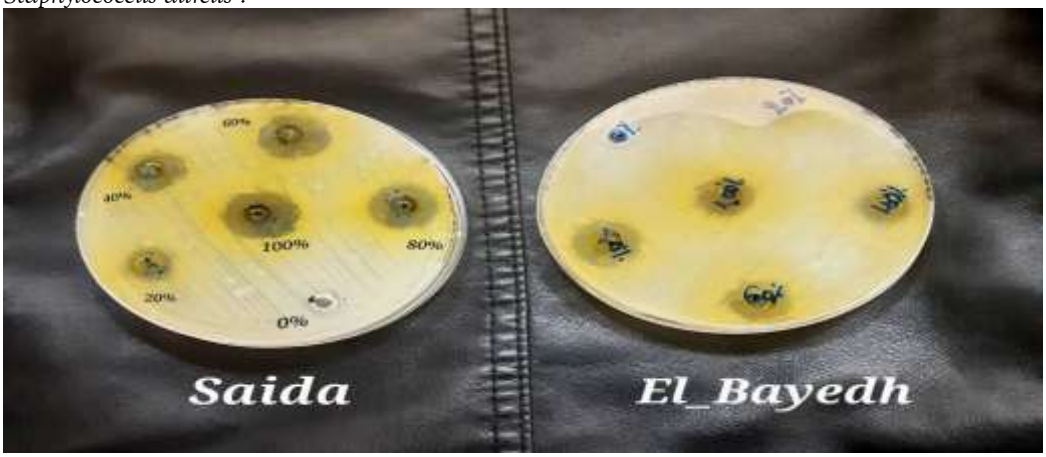

Figure 5: Antibacterial Effect of Extracts from Species of the Two Regions on *S. aureus*on *Mueller Hinton culture medium, incubated at a temperature of 37°C for 24 hours.*

After incubation at 37°C for 24 hours, we observed and measured the diameter of the inhibition zones around the wells for the two extracts, as represented in the table. We noted an increase in the diameters of the inhibition zones corresponding to the increase in concentration.

Table 2: Diameter of Inhibition Zones of the Two Extracts on *S. aureus*

concentration / diameter IZ	*IZ Saida (mm)*		*IZ El-bayedh (mm)*	
0%	0	-	0	-
20%	10	+	8	+

40%	14	+	13	+
60%	16	++	15	++
80%	17	++	15	++
100%	19	++	16	++

(IZ: inhibition zone) (-): Resistant (+): Sensitive (++): Very sensitive

The results reveal that the *S. aureus* bacteria are sensitive at concentrations of 20-40%, and very sensitive at concentrations of 60-80-100%. Significant diameters of the inhibition zones were recorded for the species from the Wilaya of Saida compared to those from the Wilaya of El-Bayedh.

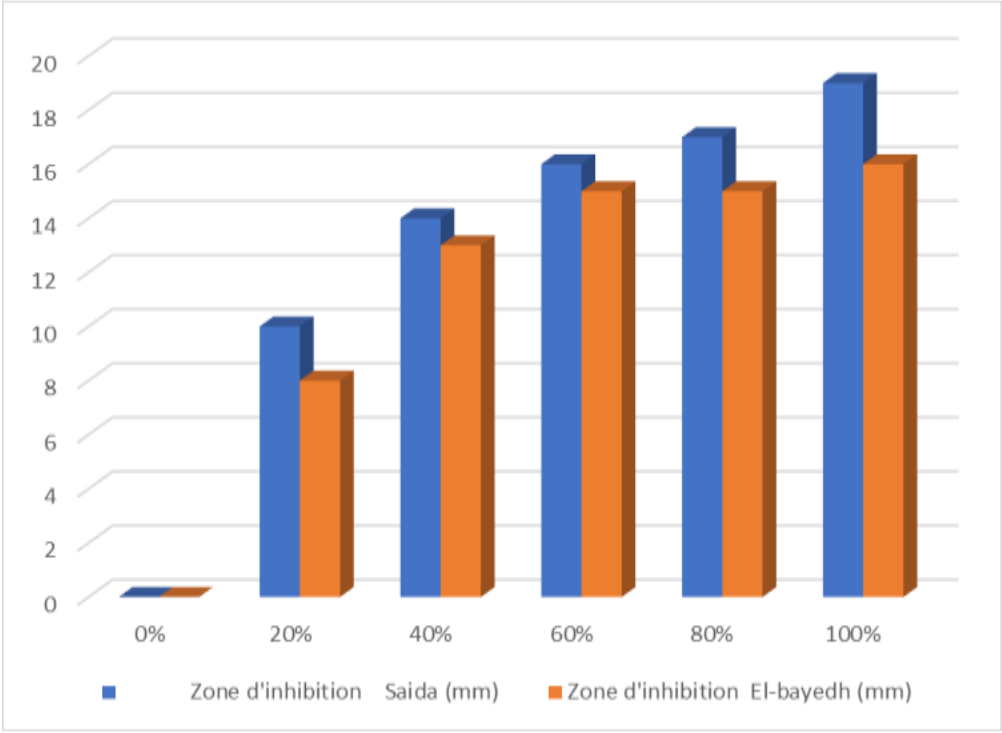

Figure 6: Inhibition Zones of the Two Extracts on *S. aureus*

Bacillus cereuse

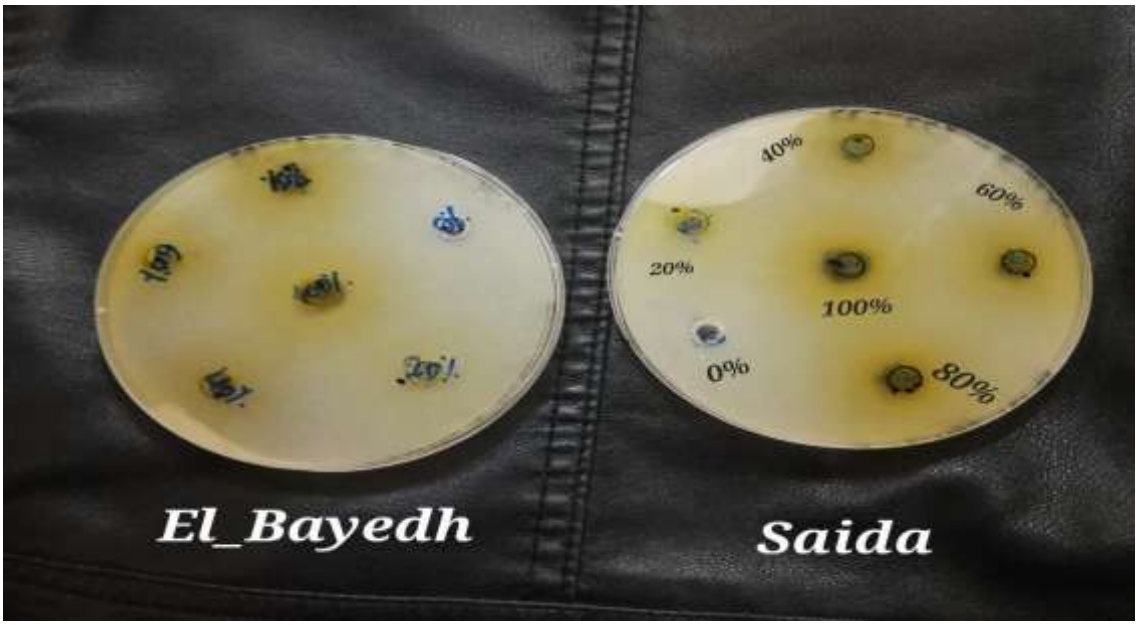

Figure 7: Antibacterial Effect of Extracts from Species of the Two Regions on *Bacillus cereus* on Mueller Hinton Culture Medium, Incubated at a Temperature of 37°C for 24 Hours.

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After incubation at 37°C for 24 hours, we observed and measured the diameter of the inhibition zones around the wells for the two extracts, as represented in the table. No inhibition zone was observed except for the concentration of 100%, where the *Bacillus cereus* bacteria were sensitive.

Table 3: Diameter of Inhibition Zones of the Two Extracts on *Bacillus cereus*

Concentration / Diameter IZ	*IZ Saida (mm)*		*IZ El-Bayedh (mm)*	
0%	0	-	0	-
20%	0	-	0	-
40%	0	-	0	-
60%	0	-	0	-
80%	0	-	0	-
100%	9	+	9	+

(IZ: inhibition zone) (-): Resistant (+): Sensitive (++): Very sensitive

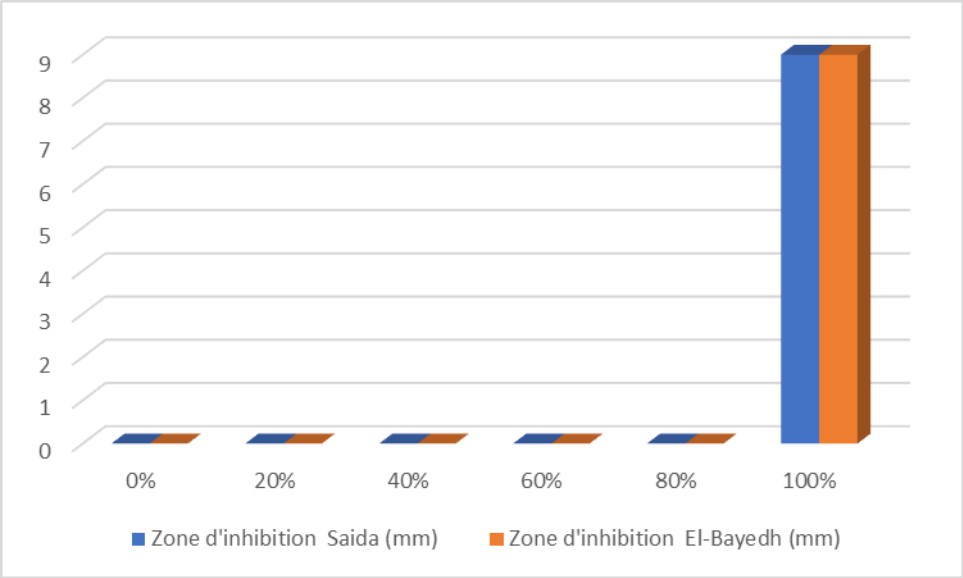

Figure 8: Inhibition Zones of the Two Extracts on *Bacillus cereus*

Escherechia coli

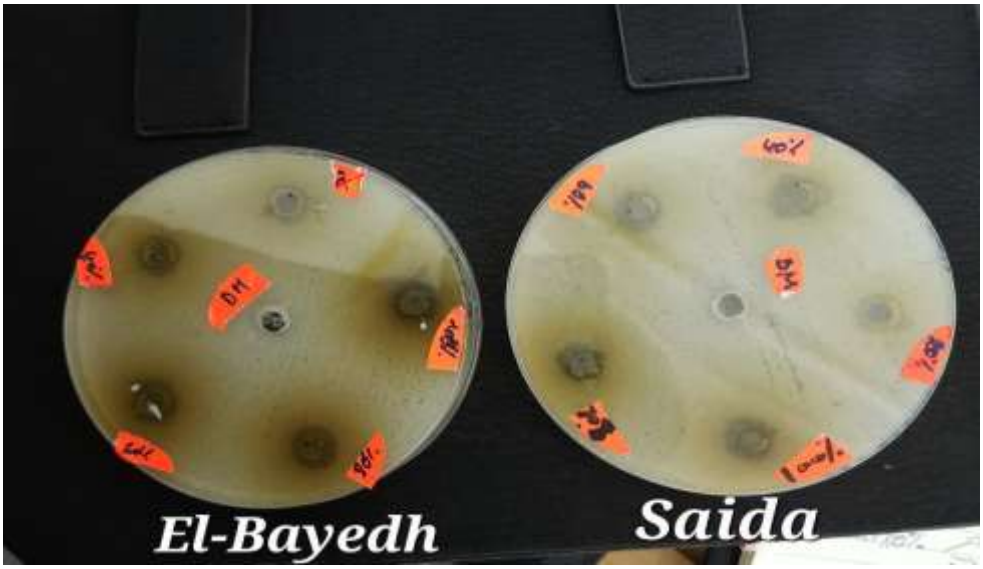

Figure 9: Antibacterial Effect of Extracts from Species of the Two Regions on *E. coli* on Mueller Hinton Culture Medium, Incubated at a Temperature of 37°C for 24 Hours.

After incubation at 37°C for 24 hours, we observed and measured the diameter of the inhibition zones around the wells for the two extracts, as represented in the table. Except for the DMSO control, we observed inhibition zones, with their diameters increasing according to the concentration.

Table 4: Diameter of Inhibition Zones of the Two Extracts on *E. coli*

Concentration / Diameter IZ	*IZ Saida (mm)*		*IZ El-Bayedh (mm)*	
0%	0	-	0	-
20%	9	+	8	+
40%	10	+	10	+
60%	10	+	11	+
80%	11	+	11	+
100%	12	+	11	+

(IZ): Inhibition Zone(-): Resistant(+): Sensitive

The results reveal that *E. coli* is sensitive to the concentrations of 20%, 40%, 60%, 80%, and 100%. Significant diameters of the inhibition zones were recorded for the species from the Wilaya of Saida compared to those from the Wilaya of El-Bayedh.

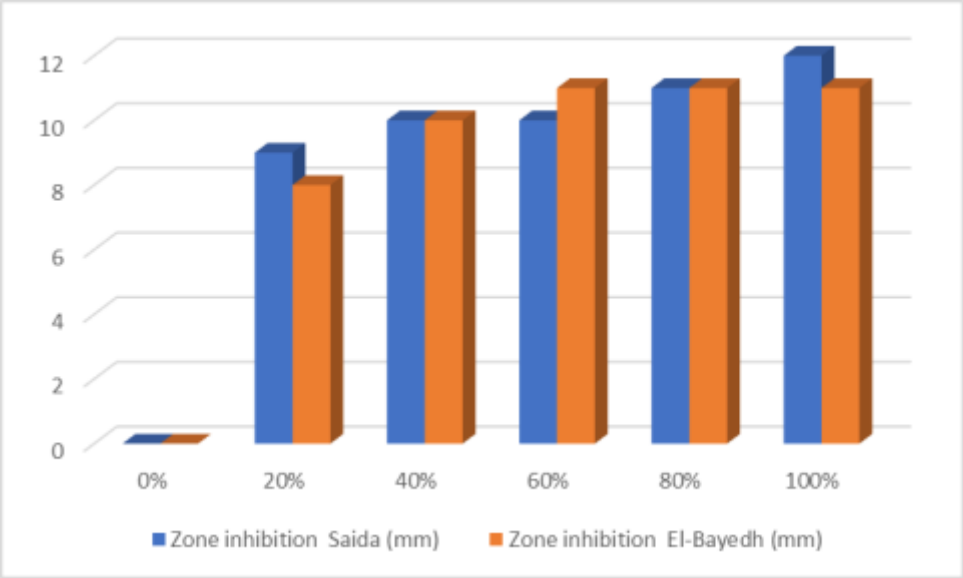

Figure 10: Inhibition Zones of the Two Extracts on *E. coli*

Candida albicans

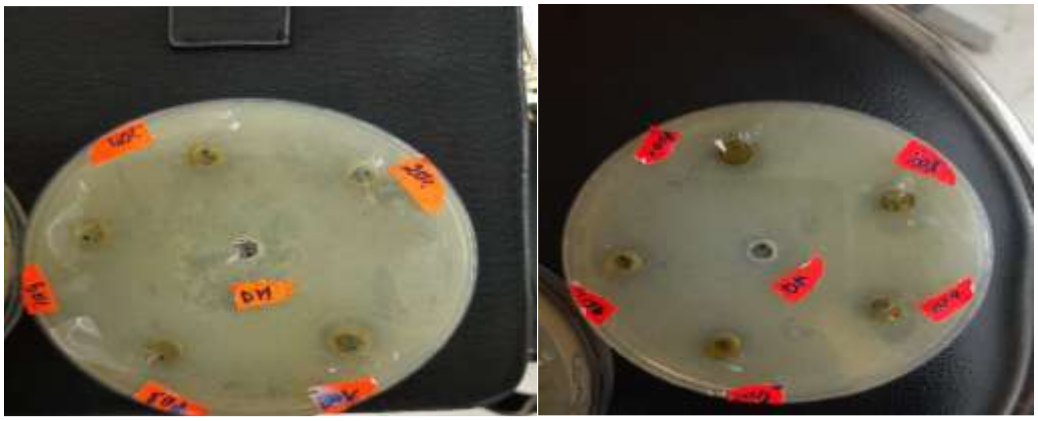

Figure 11: Antibacterial Effect of Extracts from Species of the Two Regions on *Candida albicans* on Sabouraud Culture Medium at a Temperature of 37°C for 72 Hours.

After incubation at 37°C for 78 hours, we observed and measured the diameter of the inhibition zones around the wells for the two extracts, as represented in the table. Except for the DMSO control and the 20% concentration, We observed inhibition zones, with their diameters increasing according to the other concentrations.

Table 5: Diameter of Inhibition Zones of the Two Extracts on *Candida albicans*.

Concentration / Diameter IZ	*IZ Saida (mm)*	*IZ El-Bayedh (mm)*	
0%	0	-	0 -

20%	0	-		0	-
40%	9	+		8	+
60%	10	+		8	+
80%	10	+		8	+
100%	12	+		14	+

(IZ): Inhibition Zone, (-): Resistant, (+): Sensitive

The results reveal that *Candida albicans* is sensitive to the concentrations of 40%, 60%, 80%, and 100%. Significant diameters of the inhibition zones were recorded for the species from the Wilaya of Saida compared to those from the Wilaya of El-Bayedh.

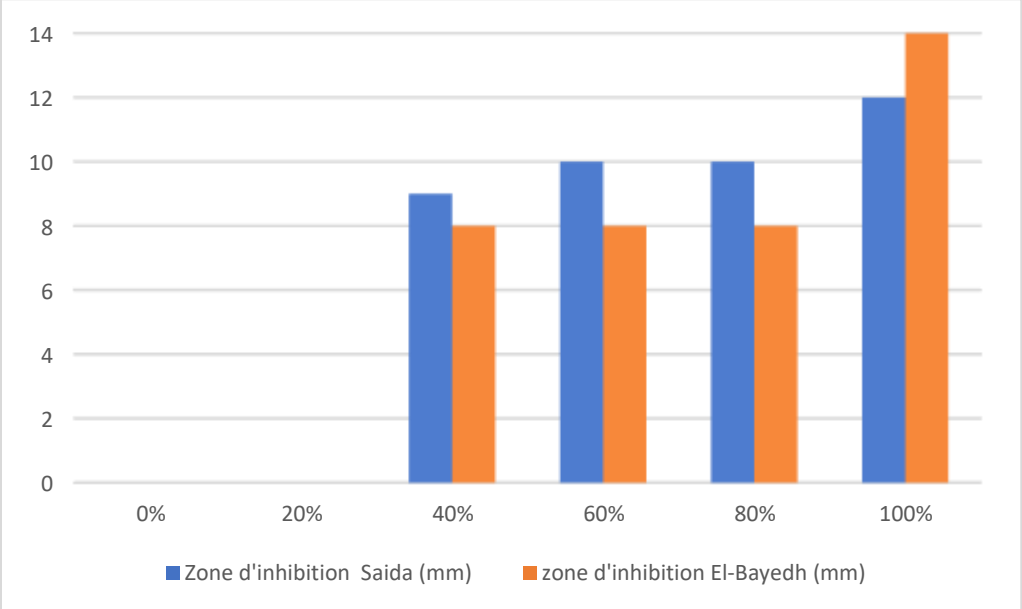

Figure 12: Inhibition Zones of the Two Extracts on *Candida albicans*

DISCUSSION

The statistical study shows that the total phenol contents differ from one extract to another. The highest contents were presented by the extract from Saida (180.16 mg EAG/g of extract), followed by the extract from El-Bayedh (138.5 mg EAG/g of extract). Our results are lower compared to another study conducted by Dorman et *al.* (2003), which showed that the aqueous extract of the aerial part of *Mentha spicata* L yielded a value of 214 mg EAG/g of extract. It is noteworthy that the total phenol content in the aqueous extract was higher than in our methanolic extract. This is evident since polyphenols are polar compounds due to their hydroxyl groups, making them soluble in polar solvents (Ignat et *al.*, 2011).Compared to the results obtained by Sweetie et *al.* (2007), the flavonoid content is 13.5 mg EQ/g of extract, which is higher than that obtained in our study (6.15 mg EQ/g MS for the extract from Saida and 5.85 mg EQ/g MS for the extract from El-Bayedh).The statistical study shows that the contents of condensed tannins in the different extracts presented significant differences. The highest contents were found in the extract from Saida (72 mg EC/g of extract), followed by the extract from El-Bayedh (57.1 mg EC/g of extract).The concentrations of total polyphenols, flavonoids, and tannins in the green mint from Saida are higher compared to those from El-Bayedh. This can be explained by differences in several parameters, including geographic, physicochemical, or biological factors such as: the harvesting site, the plant's environment, light, precipitation, topography, season, soil type, harvesting period, genetic heritage, the extraction procedure used, the part of the plant studied, or their phytochemical products.According to Cox et al. (2000), the antimicrobial activity of extracts from medicinal plants is closely related to the chemical profile of their constituents in terms of major bioactive compounds. Karmen (2015) confirms that most of these compounds possess remarkable antimicrobial properties; however, it is primarily the major volatile constituents that often exhibit inhibitory properties against microorganisms, particularly polyphenols, alkaloids, and essential oils.These compounds are molecules that belong to the secondary metabolites of plants. Approximately 1,000 substances have been characterized (Dibong et *al.*, 2011). They are essentially

the most widespread natural compounds in nature and thus undoubtedly constitute integral elements of the studied medicinal plant, *Mentha spicata*.

The tested bacterial strains appeared to be sensitive to very sensitive to the extracts from Saida and El-Bayedh at various concentrations. This clearly shows that these extracts exert antibacterial activity on the four studied strains, with a maximum inhibition diameter of 19 mm and 16 mm for *S. aureus*, 12 mm and 11 mm for *E. coli*, 14 mm and 12 mm for *Candida albicans*, and 9 mm for *Bacillus cereus* for both extracts at a concentration of 100 mg/ml.Compared to the results obtained by Barchan et *al.* (2015), the methanolic extract of *Mentha spicata* has an antibacterial effect on several bacterial strains such as *L. monocytogenes* and *E. hirae*, with no effect on *S. aureus*. In contrast, our results show that *S. aureus* is the most sensitive to the extracts of our plant. For the hexanic extract, *Mentha spicata* proved to be the most active inhibitor against *S. aureus*, *L. monocytogenes*, *E. coli* 405, *E. coli* 471, and *E. hirae*(Barchan et *al.*, 2015).Our results show good antibacterial activity of *Mentha spicata* L. against *E. coli* and *S. aureus*, comparable to the results obtained by Chama et al. (2023) with *Mentha pulegium* against *E. coli* and *S. aureus,*According to the results of the preliminary antimicrobial tests conducted, it can be assumed that the levels of polyphenols in the extracts vary proportionally with the concentration and polarity of the extraction solvent. Additionally, geographic, physicochemical, or biological parameters influence the plant used. This is evidenced by the higher inhibition efficacy of the Saida extract against the germs compared to that of the El-Bayedh region.

La Menthe verte des régions de Saida et d'El-Bayedh semble contenir l'essentiel des composés bioactifs capables de se substituer aux traitements conventionnels à base, notamment d'antibiotiques dont l'usage abusif peut développer une certaine résistance des germes nuisibles et induire par voie de conséquence des effets néfastes sur la santé humaine.

CONCLUSION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Moreover, the use of plant-based treatments and the search for new substances with biological activities are significant scientific concerns. *Mentha spicata* L is a plant that has been extensively studied for its medicinal properties due to its richness in phenolic compounds and essential oils.

In this research, we focused on the phytochemical study and antibacterial power of extracts from *Mentha spicata* L from the regions of Saida and El-Bayedh. The choice of this plant is based on some ethnopharmacological data (intestinal disorders, ulcers, microbial infections, etc.).

The antibacterial activity is significant, which explains the efficacy of the Saida extract compared to the El-Bayedh extract.

The biotope of *Mentha spicata* L is one of the factors that affect its bioactive compounds and, consequently, its biological activities such as antibacterial activity.

Medicinal plants are an important source for reducing the increase in antibiotic resistance and infectious diseases, associated with the side effects of antibiotics.

Our study resulted in the following findings:

The extraction of bioactive molecules by maceration from the leaves of the chosen species yielded an acceptable and satisfactory yield of 26.25% and 28.75%.

The evaluation of total phenol content indicates that the plant from each region is very rich in total phenols, with contents of 180.16 and 138.5 mg EAG of extract.

Regarding flavonoid content, the leaves of *Mentha spicata* L showed levels of 6.15 and 5.1 mg EQ/g of extract for each region.

The extracts of *Mentha spicata* L revealed moderate levels of condensed tannins at 72 and 57.83 mg EC/g of extract.

The antibacterial activity test of *Mentha spicata* L on the four strains shows that:

S. aureus is sensitive to concentrations of 20% and 40%, and very sensitive to concentrations of 60%, 80%, and 100%. Significant diameters of inhibition zones were recorded for the species from the Wilaya of Saida compared to those from the Wilaya of El-Bayedh.

Bacillus cereus is sensitive to the concentration of 100%.

The results reveal that *E. coli* is sensitive to concentrations of 20%, 40%, 60%, 80%, and 100%.

Candida albicans is sensitive to concentrations of 40%, 60%, 80%, and 100%.

There is a direct relationship where the higher the concentration of total polyphenols, the greater the antibacterial activity, which explains the efficacy of the Saida extract compared to the El-Bayedh extract.

The biotope of the plant *Mentha spicata* L is one of the factors that affects its bioactive compounds and, consequently, its biological activities such as antibacterial activity. Medicinal plants are an important source for reducing the increase in antibiotic resistance and infectious diseases, which are associated with the side effects of antibiotics.

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