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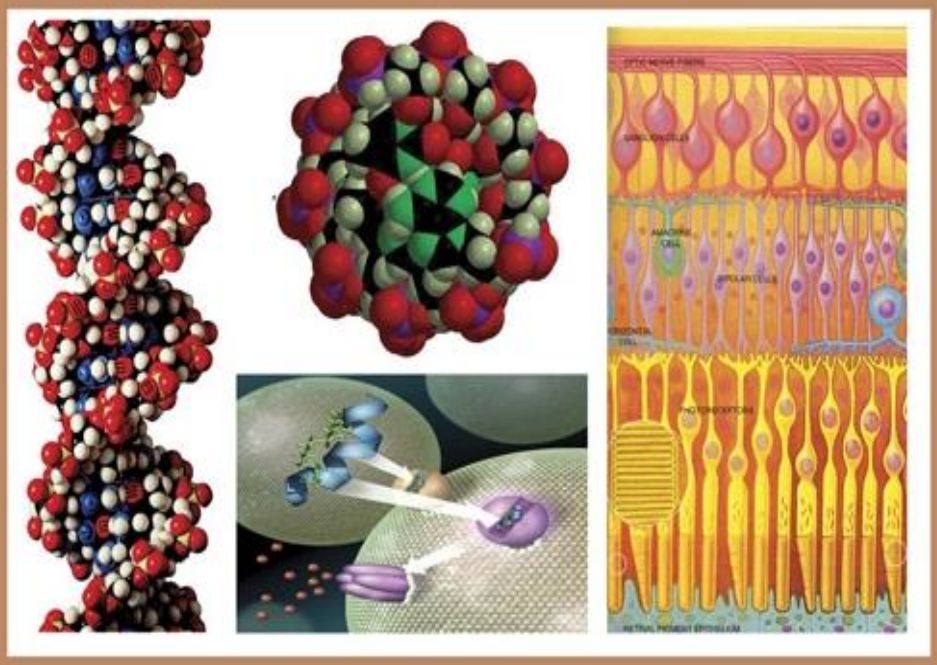

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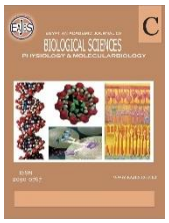

Total Phenolic Content and Antimicrobial Activity of *Mentha rotundifolia* L. from Western Algeria

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ABSTRACT

The objective of the present study is to the total phenolic compound determination, as well as the in vitro evaluation of *methanolic* and *ethanolic extracts* antimicrobial activity of the medicinal plant *Mentha rotundifolia* L. against pathogenic strains. The Follin-Cioacalteu method was used to determine the total polyphenols, while the flavonoids were estimated according to the colorimetric method with aluminum chloride. In addition, the determination of condensed tannins is performed by the vanillin method. The antimicrobial activity is tested on five pathogenic bacterial strains, two of which are Gram-negative (*Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), three Gram-positive (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923 and *Micrococcus leutus* ATCC 4698), and one yeast (*Candida albicans* ATCC 26790), by the diffusion on solid medium and microdilution methods. The *ethanolic extract* of round leaf mint from the Bechar region is very rich in polyphenols and flavonoids (401.75 ± 0.32 mg GAE/g, 549.75 ± 1.23 mg QE/g) compared to the methanolic and hydroalcoholic extracts of this plant from the Sidi-Bel-Abbes region. The results of the obtained well method showed that all bacterial strains exhibited sensitivity to the *ethanolic extract* of *Mentha rotundifolia* L. from the Bechar region, with inhibition zones up to 21,67 ± 0,57 mm and minimum inhibitory concentrations (MIC) varying between 06.25 mg/ml and 100 mg/ml, The effect of the other extracts on the tested strains was less significant, the *methanolic extract* was the least effective. These results demonstrate that the *Mentha rotundifolia* L. *ethanolic extract* has a great potential of secondary metabolites with antimicrobial activity.

INTRODUCTION

Since antiquity, herbal medicine history has started and medicinal plants have been used as remedies to treat various diseases.

This traditional culture is inherited from our ancestors, it is the oldest and most actual medical method (Gurib, 2006). Being part of the Mediterranean region, Algeria is very well known for its varied climate and the nature of its soils, promoting the development of a diverse and rich flora of medicinal and aromatic plants, from which the choice is made on a species of the *Lamiaceae* family: *Mentha rotundifolia* L. known locally as *Timarssat* or *Domrane*.

Because of the form of its leaves, its identification is not difficult, its leaves are round, thick, and wrinkled with stems characteristic of the *Lamiaceae* and with whitish bristles which cover all the aerial part (Harley, 1972). This plant colonizes wetlands and waters borders and edges, herbaceous, vivacious and very fragrant, generally used in culinary preparations, herbaceous, vivacious and very fragrant, generally used in culinary preparations, in the pharmaceutical field and even in cosmetics, it is very rich in phenolic components with antioxidant, anti-inflammatory and antimicrobial properties (Benabdallah *et al.*, 2016; Riahi *et al.*, 2018; Alharbi *et al.*, 2022). Many research studies have characterized the chemical composition and biological activities of this Algerian plant essential oils (Brada *et al.*, 2007; El Arch *et al.*, 2003), but the antimicrobial activity evaluation of the *polyphenolic extracts* and their action modes against pathogenic microorganisms have been poorly discussed. The polyphenols are the products of the plant's secondary metabolism, they are present in all these plants parts of which the total phenols, the flavonoids and the tannins are the most remarkable (Boizot and Charpentier, 2006) The objective of this research is the determination of some plant extracts secondary metabolites content from two extremely different regions: Sidi-Bel-Abbes and Bechar, and to evaluate the *in vitro* antibacterial and anticandidal activity of this plant by comparing the different results obtained.

MATERIALS AND METHODS

Plant Material:

The selected species *Mentha rotundifolia* L. was collected between March and April 2021 from two regions of western Algeria (Sidi-Bel-Abbes and Bechar) (Fig.1), the botanical identification of this plant was performed by the research laboratory of Biodiversity Plant: Conservation and Valorization at the Natural and Life Sciences Faculty in the Djillali Liabes University of Sidi-Bel-Abbes, Algeria.

Fig.1: Sampling sites from Sidi-Bel-Abbes and Bechar region. **(Google Maps, 2021).**

The leaves (Fig.2), were washed then air-dried, protected from light and humidity for one week, finely powdered and stored in hermetically sealed bottles in order to preserve the maximum possible content of antimicrobial substances.

Fig .2: *Mentha rotundifolia* L. fresh (left) and after drying (right) **(Personal photo)**.

Strain Library Database and Cultural Media:

The present study focused on different microbial strains (ATCC), which included five bacteria: three Gram-positive *Micrococcus luteus* ATCC 4698 (*M. luteus*), *Bacillus cereus* ATCC 11778 (*B. cereus*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*) and two Gram-negative *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Escherichia coli* ATCC 25922 (*E. coli*), and one yeast *Candida albicans* ATCC 26790 (*C. albicans*). The culture media used are Muller-Hinton agar and Muller-Hinton broth for the *phenolic extracts* antimicrobial activity evaluation.

***Phenolic extract* Preparation:**

The *Mentha rotundifolia* L. plant leaves powder (20g) was extracted with 500 ml of 80% methanol and 80% ethanol, under agitation for 24 hours at room temperature and protected from light. The two *organic extracts* were then filtered and concentrated under a vacuum in a rotary evaporator (Heidolph Rotavapor), then stored in a refrigerator at +4°C to be used for secondary metabolites analysis and various microbiological analyses (Messaoud *et al.*, 2012).

Yield Extracts Calculation:

The different dry *extracts* are weighed and the yield determination was done by calculating the dry weight yield per 100g of the plant powder (Messaoud *et al.*, 2012).

Phytochemical screening

The secondary metabolite's chemical composition preliminary tests (alkaloids, tannins, flavonoids and saponosides) is carried out on the vegetable powder according to the protocols described by (Edeogal *et al.,* 2005; Bouquet, 1972) and the chemical groups' research was determined on the precipitation or characteristic staining basis.

Secondary Metabolites Determination:

Total Polyphénols Determination:

The total polyphenols content was determined spectrophotometrically based on the Folin-Ciocalteu method described by (Singleton *et al.*,1999). The protocol of (Benabdallah *et al.,* 2016) was performed

with modification. For each analysis, 500 µl of the diluted *extract* was mixed with 2 ml of 10% Folin-Ciocalteu and incubated in the dark for 5 minutes. Then 2.5 ml of sodium carbonate (7.5%) is added and a second incubation in the dark for 45 minutes in a Marie bath adjusted to 45°C was performed. The optical density is measured at 760 nm. For each sample, the experiment was repeated three times. The polyphenol contents of the different *extracts* are expressed as gallic acid equivalent per g dry matter (mg GAE/g of DM).

Total Flavonoids Determination:

The flavonoid amount was estimated by the colorimetric method using an aluminum chloride ($AlCl_3$) solution. To perform this, 500 µl of $AlCl_3$ (2% in methanol) was added to 500 µl of the diluted *extract*, followed by incubation in the dark for 40 minutes at room temperature. The absorbance was measured at 430 nm (Koolen *et al.,* 2013). Flavonoid content is expressed as rutin equivalent per gram of dry matter (mg RE /g DM).

Condensed Tannins Determination:

The condensed tannins amount present in the hydroalcoholic extracts was determined by a vanillin-HCl test according to the method described by (Benabdellah *et al.,* 2016). 12.5 µl of *extract* was added to 750 µl of vanillin (4% in methanol) and 375 µl of concentrated HCl. After 15 minutes of incubation at room temperature, the absorbance at λ_{max} = 500 nm was measured with a spectrophotometer. Results are expressed as mg catechin equivalent per gram dry matter (mg CE/g DM).

Antimicrobial Activity Evaluation:

The Inoculum Preparation:

From cryopreserved stocks, bacterial species and yeast were cultured separately, then isolated colonies from fresh pre-cultures (18-24h for bacteria and 48h for yeast) were collected and transferred into tubes containing sterile physiological water. Microbial suspensions with turbidity adjusted to 0.5 Mc Farland corresponding to 10^8 CFU/ml have been prepared, they are used in two or in 24 hours, on the condition that they are maintained at 2-8°C as defined by the (European Pharmacopeia, 2008), then a swabbing plating will be applied on Petri dishes containing Muller-Hinton agar medium (MH).

Solide Diffusion Method:

The bacteria susceptibility test was performed by the well method, each inoculum was inoculated by swabbing on MH agar, then the previously dug holes were then filled with 20 µl of each plant *extract* of the concentration ranges (100%, 75%, 50%, 25%), of which 100 mg/ml is considered as the stock solution at 100% (Gupta *et al.,* 2010; Tsirinirindravo and Andrianarisoa ,2009).

The dry *extracts* obtained were solubilized in 50% Dimethyl sulfoxide (DMSO), which was previously tested against each type of pathogenic strain studied, of which no inhibition was observed. The Petri dishes were then left for 1 hour at room temperature before they were incubated at 37°C for 18-24h and then the inhibition zone diameters were measured (Oke *et al.,* 2013). Positive control was performed at the same time, by testing the germs' sensitivity to four chemical antibiotics; Céfotaxime (CTX), Amikacine (AN), Cephalexine (CL), Tetracycline (TE).

After inoculation in MH agar medium, the antibiotics discs were aseptically deposited, then the dishes were incubated at 37°C for 24 hours and the results were read by measuring the inhibition diameters (Joffin and Leyral, 2006).

The Minimum Inhibitory Concentration (MIC)/ The Minimum Bactericidal Concentration (MBC) Determination:

In order to better evaluate the antimicrobial activity, a micro-dilution method was carried out, it consists of placing the standardized inoculum in direct contact with decreasing concentrations of plant extracts in a liquid medium, in a 96-well plate, after incubation, the MIC was visually reported in which no growth is visible (Hulin, 1998). It is necessary to determine the MBC from the wells, which represent no microbial growth, by inoculating them on

Muller-Hinton agar dishes in a single streak, the MBC is indicated by the total absence of germs (Hulin, 1998).

Statistical Analysis:

The secondary metabolites tests were performed in triplicate and the obtained results are expressed in the form of the mean ± standard deviation. The student test (t) was performed using SPSS and a $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Characteristics and yield of *Mentha rotundifolia* L. extracts

The extraction of phenolic compounds by polar organic solvents from the aerial part of the studied plant species allowed us to determine the yields of its crude *extract* (Table 1).

Table. 1: Characteristics and yield of the *Mentha rotundifolia* L. *extracts*.

Origin	Extract	Color	Physical appearance	Yield
Sidi-Bel-Abbes	Methanolic	Brown	In paste form	16,00%
	Ethanolic	Brown	Powder	18,20%
Bechar	Methanolic	Brown	In paste form	19,43%
	Ethanolic	Brown	Powder	22,30%

The *Mentha rotundifolia* L. *ethanolic extract* from the Bechar region presented the highest yield (22.30%) compared to the *methanolic extract* (19.43%) from the same region. These differences are attributed to several climatic and environmental factors, the soil nature, the temperature, the extraction method and the solvent used (Su *et al.,* 2006).

The studies of (Brahmi *et al.,* 2015) on *Mentha rotundifolia* L. showed an extraction yield of (4.6%), which is lower than the one obtained in our study and considerably higher than the one reported by (Iazzouren, 2015) obtained from the same plant which was (11.23%).

Phytochemical Screening:

The phytochemical screening revealed the richness of our plant in secondary metabolites, the results are illustrated in (Table 2).

Table. 2: Phytochemical screening of *crude extracts* from the two regions studied (Sidi-Bel-Abbes and Bechar).

Species	Origin	Extract	Poly.P	Flavo	Tan	Alka	Sapo
Mentha rotundifolia L.	Sidi-Bel-Abbès	EM	++	++	+	-	++
		EE	++	++	+	-	++
	Bechar	EM	+++	+++	++	-	++
		EE	+++	+++	+++	-	++

Poly.P: Total polyphenols, Flavo: Flavonoids, Tan: Condensed tannins, Alka: Alkaloids, Sapo: Saponosides.
ME: Methanolic extract, EE: Ethanolic extract

The polyphenols (flavonoids and tannins) and saponosides present in the aerial part of our plant indicate its richness in secondary metabolites possessing various properties including antimicrobial (Bruneton, 2009). However, alkaloids are absent and the possibility of phytotoxicity was low. These results justified its use by the local population in their daily life, in culinary preparations and traditional remedies.

Secondary Metabolites Determination:

The quantitative estimation of phenolic compounds, flavonoids and condensed tannins according to the extraction solvent and the sampling locality of the *M. rotundifolia* L. aerial part are represented in (Table 3). The concentration values are presented as mean ± (Standard Deviation) SD of three measurements.

Table. 3: Total phenol, flavonoid and condensed tannin contents of the different *extracts* of *Mentha rotundifolia* L. from the two regions studied.

Species	Origin	Extract	Polyphenol content [(a)]	Flavonoid content [(b)]	Condensed tannin content [(b)]
Mentha rotundifolia. L	Sidi-Bel –Abbès	ME	284,60 ± 1,04**	141,47 ± 0,81**	16,47 ± 0,78
		EE	255,51 ± 1,93**	60,66 ± 1,24**	79,94 ± 0,46
	Bechar	ME	355,02 ± 0,36**	549,75 ± 1,23**	19,85 ± 1,08**
		EE	401,75 ± 0,32**	665,38 ± 0,73**	122,85 ± 0,38**

*: *indicates significant values P<0.05. **: indicates very significant values P<0.01. ***: indicates highly significant values P<0.001. Means ± of three measurements were expressed. ME represents the methanolic extract. EE represents ethanolic extract*

[(a)] mg gallic acid equivalent per g dry matter (mg GAE/g DM).

[(b)] mg quercetin equivalent per g dry matter (mg QE/g DM).

ME: Methanolic extract, EE: Ethanolic extract

The highest total phenol content of the round leaf mint aerial part was found in the *ethanolic extract* of Mint from Bechar region (401.75 ± 0.32 mg GAE/g DM) , followed respectively by the *methanolic extract* of the plant collected from the same region and the mint *methanolic extract* from Sidi-Bel-Abbes (355.02 ± 0.36 mg GAE/g DM and 284.60 ± 1.04 mg GAE/g DM), We observed a remarkable variability in flavonoid and tannin contents, the highest of which were respectively those of *ethanolic extracts* of mint from Bechar region (665.38 ± 0.73 mg GAE/g DM, 122.85 ± 0.38 mg GAE/g DM).

Our study confirms the results obtained in previous works, for example (Benabdallah *et al.*, 2016) found the content of 15.10 ± 0.60 mg GAE/g DM of polyphenols, 12.30 ± 0.30 mg QE/g DM of flavonoids and 3.05 ± 0.14 mg QE/g DM of tannins in the *methanolic extract* of Mint from EL Taref region of Algeria.

In addition, lower values in secondary metabolites were reported by (Brahmi *et al.*, 2015); 12.0 ± 0.3 mg GAE/g DM and 3,3 ± 0,1 mg QE/g DM, by (Iazzourene, 2015); 87.12 ± 2.74 mg GAE/g DM, 16.21 ± 0.41 mg QE/g DM, and (Alharbi *et al.*, 2021); 74.45 mg GAE/g DM et 29.3 mg QE/g DM, from the same plant native respectively from Bejaia, Tizi Ouzou (Algeria). and Al Kharj of Saudi Arabia.

On the other side (Ferdjioui, 2014) demonstrated a value of 168.642 ± 1.642 mg GAE/g DM of polyphenols in the *Mentha rotundifolia* L. *extract* from Setif (Algeria).

According to (Wojdylo *et al.*, 2007), the different extraction methods used and the solvents used to affect the phenolic contents and, in general, make the data difficult to compare and our results appear relatively high when compared to those obtained by the authors mentioned above. It is necessary to consider that the secondary metabolite content also varies according to several parameters such as geographical location, soil type and harvest period (Falleh *et al.*, 2008).

Antimicrobial Activity Evaluation:

The antimicrobial activity of the extracts from the two regions was evaluated on five bacterial strains and one yeast, it was determined by the well method and the interpretation was performed by measuring the inhibition zone diameters around each well, this activity can be considered if the zone is greater than or equal to 8 mm (Aligiannis *et al.*, 2001).

The results appeared very variable, they were expressed in mm, the strain tested, the extract type, the concentration used and the plant origin (Table 5, Figs. 3 and 4).

Table. 5: Inhibition zone diameters (mm) and microbial strain sensitivity.

Inhibition zone diameters (mm) and strain sensitivity														
Extracts%		*E. coli*		*S. aureus*		*B. cereus*		*P. aeruginosa*		*M. luteus*		*C. albicans*		DMSO
		D	S	D	S	D	S	D	S	D	S	D	S	
EES	100	08,00±0,00	±	14,33±1,15	+	14,00±1,00	+	09,00±1,00	+	10,00±0,00	+	07,33±1,15	±	06±00
	75	06,66±0,57	-	12,33±1,15	+	11,33±1,15	+	08,00±0,00	+	10,00±0,00	+	09,00±0,00	+	06±00
	50	06,00±0,00	-	11,00±1,00	+	10,00±0,00	+	08,67±1,15	+	07,33±0,57	±	06,00±0,00	-	06±00
	25	06,00±0,00	-	07,67±1,15	±	09,00±0,00	+	06,67±1,15	-	08,00±1,00	±	06,00±0,00	-	06±00
MES	100	09,00±0,00	+	16,00±0,00	+	14,33±0,57	+	07,67±0,57	±	07,67±0,57	±	10,00±0,00	+	06±00
	75	06,67±0,57	-	11,00±0,00	+	10,33±0,57	+	06,00±0,00	-	06,33±0,57	-	08,67±0,57	+	06±00
	50	06,67±0,57	-	11,67±1,52	+	08,67±0,57	+	06,33±0,57	-	06,00±0,00	-	08,00±1,73	±	06±00
	25	06,00±0,00	-	11,67±2,08	+	07,67±1,15	±	06,00±0,00	-	06,00±0,00	-	07,33±1,15	±	06±00
EEB	100	14,67±0,57	+	21,67±0,57	+	19,33±3,05	+	13,67±0,57	+	11,67±0,57	+	11,00±0,00	+	06±00
	75	06,00±0,00	-	20,33±2,08	+	13,00±2,00	+	11,33±0,57	+	09,33±1,15	+	06,33±0,57	-	06±00
	50	09,00±1,00	+	19,00±1,73	+	13,33±1,15	+	12,67±0,57	+	07,00±1,00	-	12,67±1,15	+	06±00
	25	06,67±0,57	-	16,00±1,00	+	10,67±0,57	+	13,00±0,00	+	08,67±1,15	+	06,33±0,57	-	06±00
MEB	100	12,00±0,00	+	18,00±0,00	+	11,67±0,57	+	08,67±0,57	+	09,33±1,15	+	08,67±1,15	+	06±00
	75	11,33±0,57	+	15,33±0,57	+	10,67±0,57	+	06,00±0,00	-	06,33±0,57	-	07,00±1,00	±	06±00
	50	08,67±1,15	+	16,67±0,57	+	09,67±1,15	+	06,00±0,00	-	07,00±1,00	-	06,33±0,57	-	06±00
	25	08,67±0,57	+	14,00±0,00	+	09,33±0,57	+	06,00±0,00	-	06,00±0,00	-	06,00±0,00	-	06±00

EES: Ethanolic Extract of Sidi-Bel-Abbes plant, MES: Methanolic extract of Sidi-Bel-Abbes plant
EEB: Ethanolic extract of Bechar plant, MEB: Methanolic extract of Bechar plant
D: Inhibition Zones Diameters , S: Sensitivity of microbial strains.
- : Resistant, ± : Intermediate, + : Sensitive.

The antimicrobial activity evaluation of *methanolic* and *ethanolic extracts* of our plant *Mentha rotundifolia* L. collected from two different regions, allowed us to classify them according to their effects, by comparing their antimicrobial potentials according to their inhibition diameters against the tested strains. The *ethanolic extract* of different *Mentha rotundifolia* L. concentrations from the Bechar region (EEB), expressed the best antimicrobial activity against all the tested bacterial strains, it exerted a strong inhibitory activity on *S. aureus* ATCC 25923 (21,67 ± 0,57 mm) and *B. cereus* ATCC 11778 (19,33 ± 3,05 mm) followed by *P. aeruginosa* ATCC 27853 (13,67 ± 0,57 mm) and a less effective activity on the other strains *E. coli ATCC* 25922 (14,67 ± 0,57 mm), *M. luteus* ATCC 4698 (11,67 ± 0,57 mm) and *C. albicans* ATCC 26790 (12,67 ± 1,15 mm).

This result confirms its polyphenol content; it has a broad spectrum of inhibition since its effect includes *C. albicans* yeast, Gram-positive and negative bacteria with important diameters of up to 22 mm.

On the other side, the remaining extracts (MEB, EES and MES) are even less active on all bacteria; their antimicrobial activities and the performed sensitivity tests allowed us to classify them according to their effects. The MEB is considered after EEB as the most active extract on Gram-positive strains; *S. aureus* ATCC 25923 and *B. cereus* ATCC 11778 with inhibition zones diameters between 14,00 ± 0,00 mm and 18,00 ± 0,00 mm, 09,33 ± 0,57 mm and 11,67±0,57 mm respectively, however it is less active on Gram-negative and *C. albicans.*

The *methanolic extract* of Sidi-Bel-Abbes mint is moderately active on the strains, it produces a remarkable activity on *S. aureus* and *B. cereus*, with inhibition zones of 16,00 ± 0,00 mm 14,33 ± 0,57 mm respectively.

At last, the EEB was classified as less effective compared to the other *extracts*,

which had the largest diameter of 14,33 ± 1,15 mm on both *S. aureus* and *B. cereus* strains. The inhibitory capacity of the two *extract* types of our plant collected from the Sidi-Bel-Abbes region remains the lowest compared to the *hydroalcoholic extracts* of the Bechar round leaf mint.

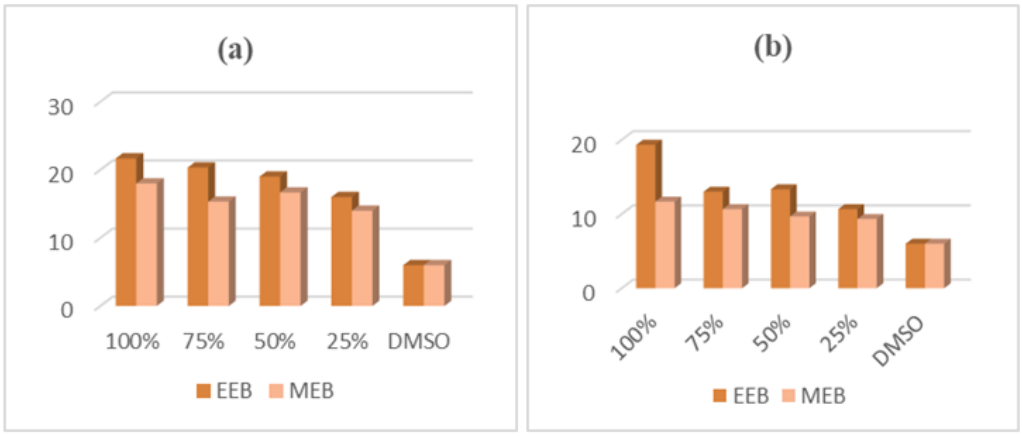

Fig 3: MEB and the EEB antibacterial activity against *S. aureus* **(a)** and *B. cereus* **(b)**.

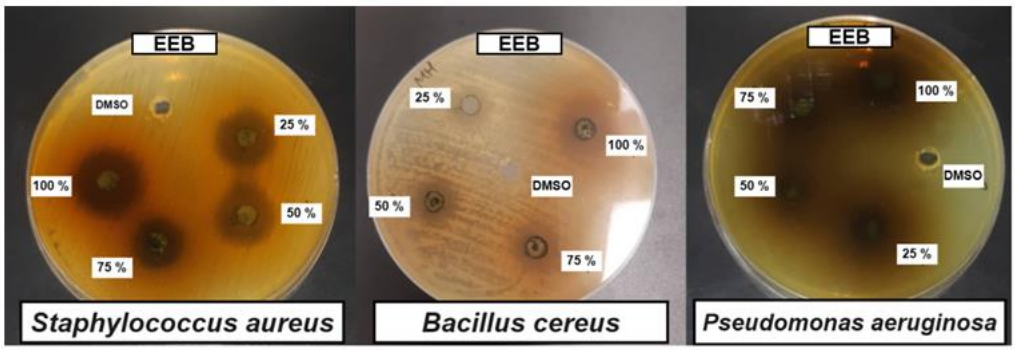

Fig. 4: EEB inhibitory effects against *S. aureus*, *B. cereus*, and *P. aeruginosa* **(Personal photo).**

In previously published articles, limited information was available on the antimicrobial activity study of this plant, which inspired us to evaluate the inhibitory power of this Algerian patrimony.

Recently, according to (Alharbi *et al.*, 2021), the active principles of *Mentha rotundifolia* L. *extracts* from Saudi Arabia are highly active on the same types of tested microorganisms (*S. aureus, E. coli, P. aeruginosa, B. cereus* and *C. albicans*) and their results are in accordance with those reported in our study. This antimicrobial activity is conferred by active biomolecules detected and characterized by HPLC in his study.

In addition, phenolic compounds extracted from the same plant originated from the Tlemcen region of Algeria in a study presented by (Seladji *et al.*, 2014) confirmed its antibacterial activity, including 30 mg/ml of its *methanolic extract* was tested on several bacterial strains which inhibition diameters varied between 6.1 mm and 10 mm. Our *extracts* remained the most active with dilutions of up to 25 mg/ml. This activity variability of the active agents depends on the chemical composition and climatic conditions of the study region.

Several researchers have demonstrated the sensitivity of Gram-positive bacteria compared to Gram-negative bacteria due to their structures and permeability to hydrophobic bioactive molecules (Shan *et al.*, 2007; Turkmen *et al.*, 2007).

The plant origin and the southern climatic conditions affect the antimicrobial activity and the ethanol used during the extraction was the best solvent. These results

were also reported by (Mohsen and Ammar, 2009) who demonstrated that polar organic solvents are used to extract the totality of phenolic compounds, however, apolar solvents allow the extraction of flavonoids and tannins (Bouterfas, 2015). According to (Gulluce *et al.*, 2006) the *methanolic extract* of the *Mentha longifolia*. s*pp* from Turkey showed no inhibition zone, it is considered as a non-active extract on 15 bacterial and 14 fungal strains based on the method followed, the preparation of the *extract* stock solution and the culture media used.

In order to compare the antimicrobial effects of the *hydroalcoholic extracts* of our plant, control antibiotics were used; Céfotaxime, Amikacine, Cephalexine and Tetracycline.

The results of the positive controls are classified according to the antibiotics tested on the same pathogenic strains and illustrated in (Table 6).

Table. 6: Sensitivity of the tested strains to antibiotics.

Strain	Antibiotics, inhibition zone diameters (mm).			
	CTX	AN	CL	TE
E. coli	07,00±0,00	19,00±1,00	19,33±1,15	13,33±1,15
S. aureus	07,66±0,57	19,33±0,57	26,33±0,57	18,66±1,15
B. cereus	09,00±0,00	17,33±0,57	19,00±1,00	11,33±0,57
P. aeruginosa	06,00±0,00	17,33± 0,57	07,00±0,00	06,66 ±0,57
M. luteus	06,00±0,00	22,33 ±0,57	22,66±1,52	15,00±0,00

CTX: Céfotaxime, AN: Amikacine, CL: Cephalexine, TE: Tetracycline.

The antibiogram revealed that all the strains tested are highly sensitive to the antibiotics AN, CL and TE, with inhibition zones ranging from 11,33 ± 0,57 mm to 26,33 ± 0,57 mm, with the exception of the *P. aeruginosa* strain which represents a strong resistance to the antibiotics CL, TE and CTX. On the contrary, *E. coli*, *P. aeruginosa* and *M. luteus* are totally resistant to Cefotaxin followed by *S. aureus* and *B. cereus* which are intermediate. The MIC/MBC results are reported in the table below (Table 7).

Table 7: Determination of MICs and MBCs of different *extracts* of the *Mentha rotundifolia* L.

Minimum inhibitory (MIC) and minimum bactericide concentration (MBC) of *ethanolic* and *methanolic extract* (mg/ml).												
Extract Souche	EES			MES			EEB			MEB		
	MIC	MBC	BP	MIC	MBC	BP	MIC	MBC	BP	MIC	MBC	BP
E. coli	100	100	1*	12.5	50	4*	12.5	100	8	12.5	100	8
S. aureus	12.5	25	2*	25	25	1*	50	100	2*	25	50	2*
B. cereus	12.5	12.5	1*	25	25	1*	100	100	1*	100	100	1*
P. aeruginosa	25	50	2*	50	100	2*	12.5	50	4	50	100	2*
M. luteus	25	50	2*	50	100	2*	06.25	25	4	25	100	4
C. albicans	25	50	2*	25	50	2*	50	50	1*	25	100	4

BP: MBC/MIC, BP with*: Bacteriostatic Power, BA with**: Bactericidal Power

From the analysis of the results in Table,07, it appears that the MICs values obtained by *ethanolic* and *methanolic extracts* against the tested microbial strains varied between 06.25 and 100 mg/ml, we noted then a very important inhibition by comparing our minimum inhibiting concentrations with the classification

described by (Aligiannis *et al.,* 2001); as follows: (MIC < 500 mg/ml), that indicates strong inhibition, (500 > MIC > 1500 mg/ml), that indicates a moderate inhibition and (MIC > 1600 mg/ml), which is considered a weak inhibition.

EEB strongly inhibits the *M. luteus*, *P. aeruginosa* and *C. albicans* strain with MICs values equal to 06.25 mg/ml and 12.5 mg/ml respectively. This is in agreement with recent studies that demonstrated the antimicrobial activity of *Mentha rotundifolia* L. with MICs similar to that reported in our study (Alharbi *et al.,* 2021).

Conclusion

Our study consisted of the evaluation of the polyphenols content, flavonoids and tannins; and the antimicrobial activity of the *methanolic* and *ethanolic extracts* of the round-leaves mint, which is a species widely used by the natives of the region during its flowering period (spring generally). The obtained results allowed us to perform a comparison between the *crude extracts* of the medicinal plant *Mentha rotundifolia* L. originating from two extremely different regions one is located in the north-west; Sidi-Bel-Abbes and the other in the south-west; Bechar, and an important variability in the content of secondary metabolites was reported and their reaction against the tested pathogenic strains. This plant is considered a natural source of phenolic compounds of which the *methanolic extract* of the species collected in Bechar provided the most important value in active substances with antimicrobial power. At last, our results revealed that the antimicrobial activity was due to the secondary metabolites contained in the aerial part of the *Mentha rotundifolia* L. plant.

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