Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(6):90-95

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antioxidant activity and exopolysaccharide-expression of pathogenic bacteria treated with *Mentha viridis* extracts

N. Chelli-Chentouf[a*], A. Tirtouil Meddah[a], C. Mullié[b], M. Bouachria[a] and B. Meddah[a]

[a]*Laboratoire of Bioconversion, Génie Microbiologique et Sécurité Sanitaire. Département de Biologie. Faculté des Sciences de la Nature et de la Vie, Université de Mascara. Route Mamounia, Mascara, Algérie*
[b]*Laboratoire de Glycochimie, des Antimicrobiens et des Agrores sources (LG2A) FRE-CNRS. Amiens Cedex 1. France*

ABSTRACT

Natural extracts contain a variety of phenolic compounds which have an antioxidant and antibacterial capacity; they can successfully replace antibiotics that show their inefficiencies against multi-resistant bacteria. In this context, this study was intended as a contribution to the understanding and evaluation of the bioactive potential of methanolic, hydro-methanolic and aqueous extracts of Mentha viridis. The qualitative analysis revealed the presence of polyphenols and flavonoids, which were confirmed-by-quantitative-analysis. The extracts of Mentha viridis expressed an important anti-production activity of the Exopolysaccharides by all pathogens strains. The amount of exopolysaccharides (EPS) produces by bacteria decreased considerably with all extracts comparatively to control (without extracts). The extracts showed strong properties to trap molecules of free radical DPPH with an inhibition power ranging from 45 to 85%.

Key words: antibacterial, antioxidant, extracts, *Mentha viridis*, multi-resistant bacteria,

---

INTRODUCTION

The infantile gastroenteritis and food poisonings represent major health problems, although drugs exist to treat these diseases, those are not always effective; because of appearance of bacteria resistant to antibiotics and toxicity of the products [1]. Since thousands of years, humanity used various plants found in its environment, in order to treat and to look after all kinds of diseases, these plants represent an immense tank of potential compounds allotted to the secondary metabolites which have the advantage of being of a great diversity of chemical structure and they have very a wide range of activities biological. The search for new therapeutic molecules equipped with antibacterial and antioxidant activity proves consequently necessity to return towards the naturalness became more than essential [1]. The role of phenolic acids, their derivatives and flavonoids involves the ability to scavenge radicals through electron transfer with reactive oxygen species (ROS). However the evaluation of these activities remains a very interesting task which can *in vivo* make the interest of many studies like *in vitro* [2]. In this context this present research task fits, of which the principal goal is to study the antibacterial and antioxidant activities *in vitro* of various extracts and essential oil of *Mentha viridis*: plant largely used throughout the world; there exists more than thousand two hundred varieties cultivated out of the five continents, it belongs has the family of the Lamiaceae and it present many virtues [1]. Because of the importance of the bacterial adhesion - which constitutes a stage necessary for the gastro-intestinal colonization of the tract, the aim of this study was to evaluate *in vitro* antioxidant activity and the exopolysaccharide's production of multiresistant bacteria responsible of gastroenteritis and food poisoning, in presence of *Mentha viridis* extracts.

EXPERIMENTAL SECTION

Preparation of the extracts

The pulverized plant material was cold extracted in methanol (20 g of fine powder in 200 ml of methanol) for methanolic extract, another 20g was extracted in mixture of methanol/water (80/20) (v/v) to constitute hydromethanolic extract and the third one 20 g of plant material was extracted in water for aqueous extract [3].The separated extracted were then filtered through Whatman's No. 1 filter paper and the methanol filtrate were separately concentrated to dryness in vacuo using a rotary evaporator (at 60°c) to remove the methanol. The aqueous extract was lyophilized to obtain a dry powder extract [4]

Total phenolics content

The amount of total polyphenols was determined using the Folin–Ciocalteu's method. Briefly, 1 ml of the methanolic extract was mixed with 1 ml of 1/10th Folin–Ciocalteu reagent. After 5 min, 10 ml of aqueous Na2CO3 (7%, w/v) were added. The mixture was allowed to stand for 90 min at 23 1C and then absorbance was read at 750 nm (JENWAY IC 6400 UV/ visible). A standard curve was prepared using gallic acid over a range of 0 to 1 mg/ml. Total polyphenols values are expressed in gallic acid equivalents (GAE) per gram of dry weight (mg GAE g^{-1} DW) [5].

Total Flavonoids Content

Method of the aluminium trichloride ($AlCl_3$) [6] is employed to determine the content of flavonoid total in the various extracts. A calibration curve (y= ax+b) established by the catechine (0-40ug/ml), realized under the same operating conditions that the samples, will be used for the quantification of the flavonoid. The content of flavonoid is expressed in microgram of equivalent of catechine per milligram of extract [6].

Antioxidant activity: DPPH radical scavenging assay:

The antioxidant activity of the *M. viridis* extracts were assessed by measuring their scavenging abilities to 2,2´-diphenyl-1-picrylhydrazyl stable radicals. The DPPH assay was performed as described by Gachkar et *al*. [7]. 100µl of samples (studied extract) from 100µg/ml to 1000µg/ml were mixed with 2.9ml of DPPH solution (2.4mg in 100mL of MeOH). After 30min of incubation at a room temperature, the absorbance of the reaction mixture was measured at 517 nm. Scavenging of DPPH free radical in percent (%) was calculated in following way:

DPPH radical scavenging (%) = $100 \times (A_{blank}\text{-}A_{sample}/A_{blank})$ where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds and A_{sample} is the absorbance of the test samples. IC50 values, which represented the concentration of studied extracts that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

Bacterial strains

Pathogenic bacteria frequently implied in infantile gastroenteritis: *Clostridium difficile* isolated from beef meat and the cow's milk, along with *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella oxytoca*, strains isolated from ill children's stools were provided by the Laboratory of Medical Analysis in the Wilaya of Mascara. Strains were maintained on nutrient agar slopes at +4°C. Before experimental use, cultures were subcultured twice in nutrient broth (peptone 15.0 g, yeast extract 3.0 g, sodium chloride 6.0 g, D (+) glucose 1.0 g, distilled water 1 L)

In vitro* evaluation of antibacterial activities of *Mentha viridis

1. Antibiogram (method of Diffusion agar)

Inoculation of 10^6cfu/ml of bacterial culture of each strain sown on the agar surface, after incubation at 37°C during 24h ; we proceed to the determination of the diameter of inhibition zone (expressed in mm) formed around each disc [8]. The antibiotics used are: colistin (CT), aztreonam (ATM), gentamicin (CN), oxacillin (OX), cefazolin (CZ) and the spiramycin (SP).

2. Exopolysaccharides Content (EPS)

Immobilization of bacteria was done by exposing the suspensions bacteria to the ultrasounds waves (52 khz /10 minutes). Add the extracts. After incubation at 37°C for 24h, the extraction of the exopolysaccharides (EPS) was carried out [9]. The cells were harvested by centrifugation at 5000g/15min after boiling at 80°C, precipitate the EPS. Filter the supernatant at 4°C. Add three volumes of ethanol, then centrifuge at 10.000g /20min at 4°C.Resuspended in sterile physiological water. The quantification of exopolysaccharides (EPS) was carried out by the essay of total sugars described by [10]. Add to 1ml solution of EPS, 1ml of phenol and 5ml of sulfuric acid. The absorbance (A) was measured after vortexing the mix at 490nm comparing to control (without extract).

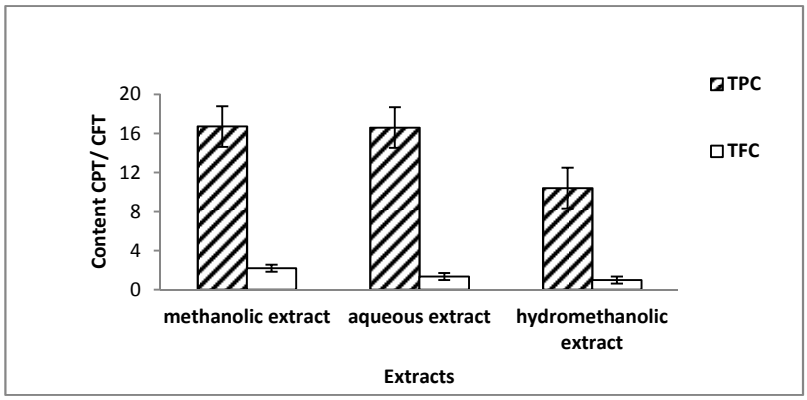
Statistical analysis
All experiments were made in duplicate. All data are presented as means ±SD.

RESULTS AND DISCUSSION

Yield
The greatest yield was observed with the aqueous extract (10%), followed by the methanolic extract (9%) then the hydro-methanolic extract (6%) respectively.

Total polyphenols Content
The content of phenolic compounds of each extract was calculated and expressed in milligrams of equivalent gallic acid per gram of dry weight (mg EGA/g DW).The total polyphenols content of the various extracts shows that the methanolic and aqueous extract presents a high content 16.72 ± 6.10 mg EGA/g DW and 16.61 ± 0.51 mg EGA/g DW respectively followed by the hydro-methanolic extract 10.40 ± 2.22 mg EAG/g DW (fig. 1). The extracts of *Mentha pulegium*, showed that the total polyphenols content borders 15% [11]. The distribution of the secondary metabolites can change during the development of the plant. This can be related to the hard climatic conditions (the high temperature, solar exposure, drought, salinity), which stimulate the biosynthesis of the secondary metabolites such as the polyphenols [12]. Indeed, the phenolic content of a plant depends on a certain number of intrinsic factors (genetic) and extrinsic (climatic conditions, cultural practices, maturity with harvest and conditions of storage) [12]. The solvent of extraction carries nonphenolic substances like sugars, the proteins and them dyes which can interfere during any phenolic evaluation [13].

Total Flavonoid content
The content of the flavonoid was carried out according to the trichloride method of aluminium by using like standard the catechine. The content of flavonoid is expressed in milligram of equivalent of catechine per gram of extract (mg EC/g DW). The methanolic extract is also richest in flavonoid with 2.2. ± 0.06 mg EC/g of extract followed by the aqueous extract with 1.35 ± 0.04 Mg EC/g and the hydro-methanolic extract with 0.98 ± 0.02 mg EC/g of extract (fig.1). *Mentha pulegium* of southern Spanish obtained either 28mg± 0.06EC/g for the alcoholic extract or 24 ± 0.01 for the aqueous extract while the contents approach for the extract hydo-alcoholic [14]. The explanation of this difference concerning the rate of the flavonoid, can lie in made that they are synthesized in abundance at certain parasitized plants, to push back certain insects by their unpleasant tastes or to protect themselves from the attack from a close plant [15]. However, it is difficult to compare these results with those of the bibliography because the use of various methods of extraction and the differences climatic and the conditions in storage, reduced the reliability of a comparison between the studies [15].



TPC:Total polyphenol content ,TFC: total flavonoid content, mg: milligram, EGA: equivalent gallic acid, DW: dry weight
Fig.1Total polyphenol and flavonoid content in the extracts of *M. viridis*

In *vitro* antioxidant activity of *Mentha viridis* (reduction of DPPH)
The antioxidant activity of extracts of *M. viridis* tested with radical DPPH was evaluated by spectrophotometer with 517 nm by comparing it with the vitamin E and while following the reduction of the DPPH which is accompanied by its passage by the color violet to the yellow color. The variation of the power of reduction according to the concentration of the extracts enables us to calculate IC_{50} parameter (the effective concentration of the substrate (extracts), necessary to reduce 50% of the free radicals in the reactional medium). The IC_{50} values were calculated graphically; where the X-coordinate is represented by the concentration of the compounds tested and the ordinate by the power of reduction expressed as a percentage [16]. Among the three extracts of *Mentha viridis*, the aqueous extract represents the most active extract with a PR of 68% (of 400 with 1000ug/ml) and an IC_{50} of 440

ug/ml, followed by the methanolic extract with a PR of 60% (800 and 1000ug/ml) and an IC_{50} of 650ug/ml and in the last, the hydromethanolic extract with a PR about 43% (800 and 1000ug/ml) and IC_{50} of 950ug/ml (fig.2). It seems according to these results that the vitamin C is the most effective antioxidant compared to the extracts studied (fig. 3) with an IC_{50} of 220µg/ml and a power of reduction going until the 90.94% with 1000ug/ml.

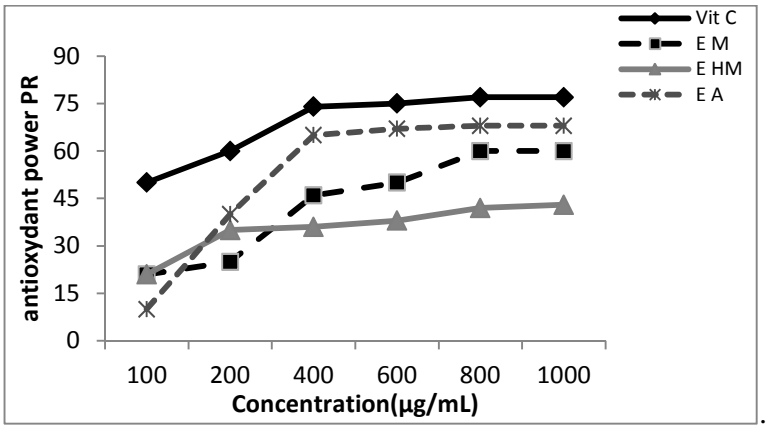
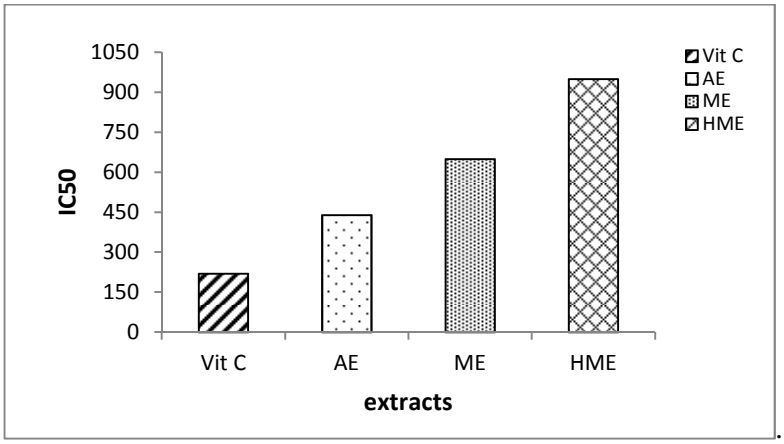


Fig. 2: antioxidant activity of studied extracts compared to vitamin C

Almost 90% of the studies on the antioxidant activity use the method of the DPPH: this method is simple but strongly sensitive [17].It is reported that the phenolic compounds can give a hydrogen atom to the free radicals stopping of this fact the chain reaction of propagation during the process of oxidation [18]. Many studies established relations between the chemical structures of the flavonoïds and their antioxidant capacities; the activity of these molecules to trap the free radicals depends primarily on their structures. The tannin also has a radicalising action of trapping on the radical 1-1 diphényl-2-picrylhydrazyl (DPPH).



Vit C: vitamin C, AE: aqueous extract, ME: methanolic extract, HME: hydro-methanolic extract

Fig. 3: IC_{50} values of vitamin C and extracts of *M. viridis*

Results of *in vitro* antibacterial activities of *Mentha viridis*

1.Antibiogram

According to the recommendations of the committee of the antibiogram of the French company of Microbiology [19], results of this test indicates that the response to antibiotics tested varied from a bacterial strain to another (Table 1)

Table 1: Antimicrobial activity profile of selected antibiotics (expressed as diameter of inhibition zone in mm)

antibiotic / Bacteria	CT	ATM	CN	OX	CZ	SP
E. coli	S (24)	S (45)	S (35)	R (6)	R (5)	R(5)
Klesbsiella oxytoca	S (15)	R(8)	R (14)	R (8)	R (5)	R(6)
Staphylococcus aureus	R (5)	R(15)	S (20)	S (24)	S (15)	I (20)
Clostridium difficile	S (12)	R (10)	S (15)	R(7)	R(8)	R(8)

R: resistant, S: sensible, CT: colistin, ATM: aztreonam, CN: gentamycin, OX:oxacillin, CZ: cefazolin, SP: spiramycin

2. Quantification of the Exoplysaccharides without treatment

The amount of exopolysaccharids (EPS) varied from 0, 8 to 3,2 mg/l (fig. 4). *In vivo*, the production of the EPS is strongly competing; the EPS give to the bacteria a competitive advantage, to allow them to be maintained and makes them more resistant to the desiccation and the attacks by the antibiotics. It was also shown that the matrix of EPS could physically prevent the entry of some antimicrobial agents inside the biofilm, while acting like an ion exchange for example, and thus reducing the diffusion of composed since the external medium towards the interior of the biofilm [20].

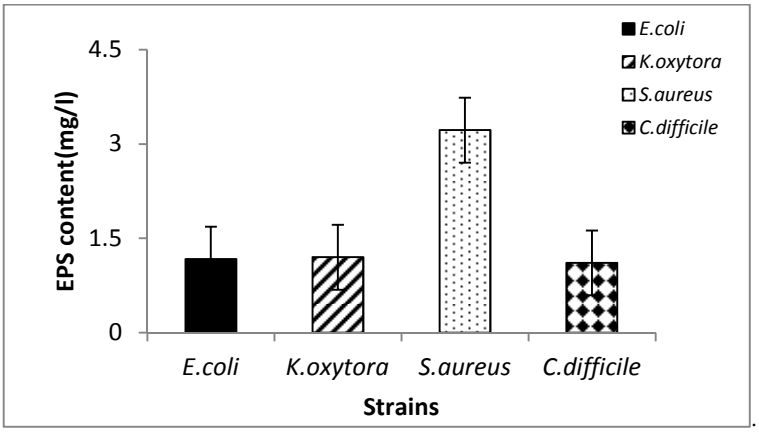
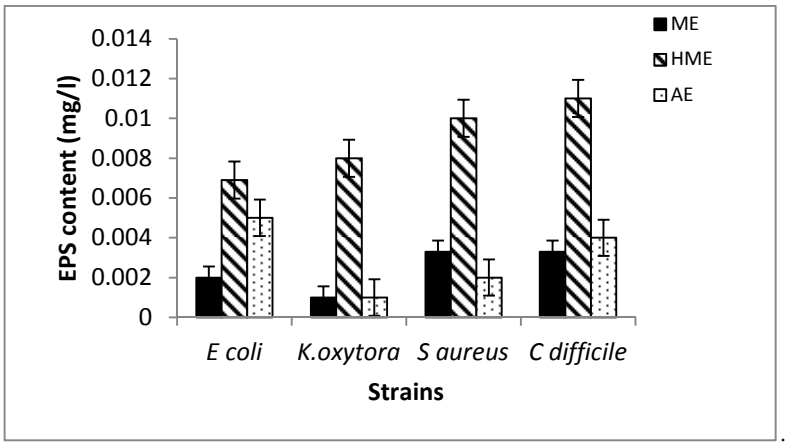


Fig.4. Exopolysaccharides content of pathogens strains

3. Quantity of the EPS from pathogens strains after treatment by *M. viridis* extracts

The extracts of *Mentha viridis* expressed an important anti-production activity of the EPS by al pathogens strains (fig.5). Various factors can affect this production such as the concentration in divalent cations, the ratio carbon-azotes and the availability in certain substrates. Indeed, the production of EPS required much energy and it thus appears improbable that bacteria used as much energy and substrate without an advantage gains [21].



EPS: exopolysaccharides, ME: methanolic extract, HME: hydromethanolic extract, AE: aqueous extract

Fig.5. Exopolysaccharides content of pathogenic strains with *Mentha viridis* extracts

CONCLUSION

The medicinal herbs constitute true chemical plants from which it is necessary to gain the maximum of profit. For obtaining various extracts, an aqueous maceration (extracted aqueous) and an organic extraction (methanolic and hydro-methanolic extracts) were carried out. Our extracts presented a remarquable yield (6, 9 and 10% for the hydro-methanolic, methanolic and aqueous extract successively).Our results indicate that *M. viridis* extracts have an important inhibiting capacity of bacterial adhesion, according to decreases of the EPS production. Extracts of *Mentha viridis* have an important antibacterial qualities; for the treatment of the gastroenteritis in the children and the infants. Also, an antiradical effect was observed with all extracts tested comparing to vitamin C. Complementary tests will be necessary and have to be able to confirm the performances put in obviousness's. We think of showing through this work which plants constitute a tank very interest for research in the future.

REFERENCES

[1] A Fournet, V Muñoz. *Current Topics in medicinal chemistry*, **2002**, 2:1213-1235.
[2] N Zeghad. Etude du contenu polyphénolique de deux plantes médicinales d'intérêt économique (*Thymus vulgaris, Rosmarinus officinalis*) et évaluation de leur activité antibactérienne.2009. mémoire de magister – université Mentouri de Constantine – Algérie.
[3] D Diallo, R Sanogo, H Yasambou, A Traoré, K Coulibaly, A Maiza. Etude des constituants des feuilles de *Ziziphus mauritiana* Lam. (Rhamnaceae) utilisées traditionnellement dans le traitement du diabète au Mali. *C. R. Chimie*, **2004**,7: 1073-1080.
[4] O O Igbinosa, E O Igbinosa, O A Aiyegoro,. *African Journal of Pharmacy and Pharmacology*, **2009**, 3, 58-62.
[5] V Dewanto, X Wu, K K Adom, RH Liu. *Journal of Agricultural and Food Chemistry*, **2002**, 50, 3010-3014.
[6] Z B Yi, Y Yu, Y Z Liang, B Zeng. *Food Science and Technology* **2007**, 4, 1000-1016.
[7] L Gachkar, D Yadegari, M B Rezaei, M Taghizadeh, S A Astaneh and I Rasooli. *Food chem*, **2007**, 102, 898-904.
[8] V Guérin-Faublée, G Carret, .L'antibiogramme : principe, méthodologie, intérêts et limites, Journées nationales GTV-INRA, **1999**, 5-12.
[9] A. Ricciardi, E. Parente, M.A. Crudele, F. Zanetti, G. Scolari and I. Mannazzu. *Journal of Applied Microbiology*, **2002**, 92, 297–306.
[10] M Dubois, A Gilles, J K Hamilton, PA Rebers, F Smith. *Anal. Chem*, **1956**, 28 (3), 350-356.
[11] S Khennouf, D Benchiekh, S Djidel, S Dahamna, S Amira, N Charef, A Baghiani, D Harzallah and L. Arrar. Polyphenols and antioxidant properties of extracts from *Mentha pulegium L.* and *Matricaria chamomilla L.* **2013**.
[12] H Falleh, R Ksouri, K Chaieb, N Karray-Bouraoui, N Trabelsi, M Boulaaba, C Abdelly. C. R. *Biologies*,**2008**, 331, 372-379.
[13] A Djeridane, M Yousfi, B Nadjemi, D Boutassouna, P Stocker, N Vidal. *Food Chemistry*,**2006**, 97(4), 654–660.
[14] M Altanassova, S Georgieva, K Ivanchiva. *Journal of University of chemical technology and metallurgy*, **2011**, 46(1), 81-88.
[15] A Goffart, M S Remacle, O Scoubeau. la nature nous en fait voir de toutes les couleurs, **2002**, Université Catholique de Louvain Festival des Sciences – UCL – Belgique.
[16] E Portes .Synthèse et étude de Tétrahydrocurcuminoïdes : propriétés photochimiques et antioxydantes, applications à la préservation de matériaux d'origine naturelle .**2008**. Thèse de doctorat. № 3695 - université Bordeaux I, 244p.
[17] JK Moon, T Shibamoto **.** Antioxidant assays for plant and food components. J Agric Food Chem, **2009**, 57(5), 1655-66.
[18] N Turkmen, Y S Velioglu, F Sari, G Polat. *Molecules*, **2007**,12, 484-496.
[19] Société Française de Microbiologie. Comite de l'antibiogramme de la Société Française de Microbiologie. Recommandations. **2010**.1-49
[20] A De Almeida Cournet. Etude de la catalyse microbienne de la réduction électrochimique du dioxygène **2010**. Thèse de doctorat .Université de Toulouse III, France.
[21] C Mouton, J C Robert. Bactériologie bucco-dentaire Chapitre 2: la plaque dentaire. Chapitre 3: acquisition de la flore buccale et formation de la plaque **1994**. Edition Masson, Paris.