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# Extraction study and the antibacterial activity of phenol and flavonoid contents in *Mentha pulegium L.* from Algeria

**A. Khadraoui [1,*], K. Hachama [2], M. Khodja [1], A. Khelifa [1], R. Mehdaoui [1], H. Harti [3], S. Abid [3], B. Agnieszka Najda [4,] N. Chahboun [5,6]**

*[1] Laboratoire de Génie Chimique, Université de Blida 1, Route de Soumaa, 09000 Blida, Algérie*
*[2] Laboratoire de la valorisation des substances naturelles, Université Djilali Bounaama, Khemis-Miliana, Algérie*
*[3] Université Djilali Bounaama, Khemis-Miliana, Algérie*
*[4] Department of Vegetable and medicinal plants, University of Life Science in Lublin, K. Leszczynskiego street 58, 20-068 Lublin, Pland.*
*[5] Laboratoire de Biotechnologie, Environnement et Qualité (LABEQ), Département de Biologie, Faculté des Sciences, Université Ibn Tofaïl, BP 133, 14000 Kenitra, Morocco.*
*[6] Laboratoire de procédés de séparation, Département de chimie, Faculté des Sciences, université Ibn Tofail 133, 14000 Kenitra, Morocco.*

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** Corresponding author. E-mail address: khadraoui.abdelkader@gmail.com*

## Abstract
The present study describes the total phenolic and flavonoids content, and in vitro antibacterial activity of petroleum ether, ethyl acetate and n-butanolic extracts from *Mentha pulegium L.* growing in Tarik Ibn Ziad within the region of Ain-Defla located in northern Algeria. The harvesting of the plant is made for three months: February, March and April 2013. We chose four bacterial strains common in human pathology, belonging to Gram positive and Gram negative classes. Bacterial strains used were: Pseudomonas aeruginosa (Gram negative), Escherichia coli (Gram negative), Klebsiella pneumoniae (Gram negative) and Staphylococcus aureus (Gram positive). These bacterial species are responsible for skin infections (Staphylococcus aureus), urinary and digestive tract infections (Escherichia coli), and nosocomial infections (Klebsiella pneumoniae and Pseudomonas aeruginosa). Antimicrobial was performed with the Bauer-Kirby method by the well diffusion technique using Petri plates made by Muller Hinton agar containing the culture. We initially made the extractions with ethanol and after we used petroleum ether, EtOAc and n-BuOH. Tests by the Shibata reaction and chromatography (TLC) showed the presence of flavonoids in all samples. The quantitative determination of total polyphenols by Folin-Ciocalteu presented the richness of ethyl acetate extract polyphenol (115.81, 277.38, and 61.36) µg GAE/g (Microgram of Gallic Acid Equivalent / gram) for crops of February, March and April respectively. Expression of results of antibacterial activity showed that the petroleum ether extract (28%) and the n-butanol extract (30.67%) are more active in Klebsciella pneumoniae; by against the ethyl acetate extract is more active on E .coli (32%).

***Keywords**: Mentha pulegium L.*, flavonoids, polyphenols, antibacterial activity, Folin-Ciocalteu, Shibata reaction.

## 1. Introduction
With its varied and usually very sunny climate, Algeria has considerable potential for medicinal and aromatic plants such as mints. This type of plant has very interesting biological properties which find applications in various areas, namely in medicine, pharmacy, cosmetics and agriculture. However, the evaluation of plant protection properties, antioxidant and antimicrobial remains a very interesting and useful task. They represent a new source of active compounds [1-7]. Indeed, the secondary metabolites are the subject of much research in vivo and in vitro, in particular, the search for new natural constituents such as phenolic compounds. Many studies have shown that flavonoids were able to inhibit different types of microorganisms: bacteria, yeasts, molds, protozoa and even viruses. However, there was high specificity between the active molecules and the target microorganisms; hence the importance of choosing the appropriate flavonoid [8-10].
Like other species of mint, used in traditional medicine; the *Mentha pulegium L.* has identical properties: digestive, carminative, cholagogue, antispasmodic, pulmonary antiseptic, refreshing, tonic, appetizer, stomachic, choleretic, expectorant and bechic. The leaves and flowering tops are used, against palpitations, intestinal fermentation, liver pain, dizziness, general weakness, hiccups, chronic bronchitis and obstinate cough [11, 12].

As part of the valuation of Algerian plant species, and given the therapeutic properties that represent Lamiaceae, we were interested in the extraction of flavonoids *Mentha pulegium L.*, from the Tarik Ibn Ziad area south of the wilaya Ain-Defla, and study especially the antibacterial activity of these extracts.

## 2. Materials and methods

### *2.1. Plant Material*

*Mentha pulegium L.* was collected from Tarik Ibn Ziad village within the region of Ain-Defla located in northern Algeria, during February to April 2013. The taxonomic identification of plant material was confirmed by Mr. Kouache Ben Moussa and a voucher specimen was deposited in the Herbarium of the agronomic department, Khemis-Miliana University, Algeria. The leaves were washed with distilled water and dried in the shade for a period ranging from 5 to 7 days at room temperature, then its were powdered by a domestic blender.

**a** ***b***

**Figure 1: (a):** fresh leaves of *Mentha pulegium L*; **(b):** dry leaves of *Mentha pulegium L*.

### *2.2. Determination of humidity of the plant*

A test sample of 20 g of leaves of *Mentha pulegium L* is disposed to be dried in the open air.
The sample is weighed daily until its weight becomes constant. The humidity level is estimated by the following formula:

$$\%H = \left( \frac{M_0 - M}{M} \right) \times 100$$

%H: Humidity level; $M_0$: weight of the sample in a fresh state in g; M: Weight of the sample after drying in g.

### *2.3. Preparation of Extracts*

A dry powder of the plant (13g) was extracted according to [13, 14], with either 250ml of ethanol-water mixture (70:30, v/v), by using Soxhlet extractor for 16h. After cooling to room temperature, the ethanolic extract is evaporated under reduced pressure using a rotary evaporator. The dry residue obtained is taken up in 30 ml of boiling distilled water and is initially mixed with 30 ml of petroleum ether in a separating funnel. After decantation of the two phases, the petroleum ether phase is recovered and the aqueous phase is again divided with 30 ml of ethyl acetate. The same procedure is repeated for n-butanol. The operation is repeated twice for each step. The phases obtained are dried using a rotary evaporator. The dry residues were taken up by a few milliliters of methanol and kept at +4°C. Finally three fractions are obtained: fraction with petroleum ether (PE), fraction with ethyl acetate (EtOAc) and fraction with n-butanol (BuOH).

### *2.4. Determination of extract yield*

The percentage yield (R %) of the extract was determined gravimetrically using the dry weight of extract (x) and soaked samples material (y) as follows :

$$\%R = \left( \frac{x}{y} \right) \times 100$$

### *2.5. Total Polyphenolic Contents (TPC)*

Total soluble polyphenolic in the different extractives of *Mentha pulegium L.* were determined with Folin Ciocalteu reagent using gallic acid as a standard [15]. Briefly, the appropriate dilutions of each extract (200 µL) were mixed with 1ml of 1:10 Folin-Ciocalteau reagent. The solutions were mixed and incubated for 4 minutes. After incubation,

800µl of $Na_2CO_3$ (75g /L) was added. The final mixture was shaken and then incubated for 2 hours in the dark at room temperature, the absorbance values at 765 nm were recorded using a UV-Vis spectrophotometer. The total phenol content of plant parts was expressed as micrograms of Gallic acid equivalents per milligram of dry weight (µg GAE/mg DW) from a calibration curve with Gallic acid (5-200µg/ml). All samples were analyzed in three replicates. The amount of total phenols is calculated by the following equation:

$$T = \frac{C \times V}{M}$$

T : Represents total phenolics (µg GAE/mg DW);
C : Ethanolic extract concentration equivalent to Gallic acid, obtained from the calibration curve (µg/ml);
V : volume of the extract (ml);
M : Dry weight of extract of each phase (g).

*2.6. Separation and identification of flavonoïds*

*2.6.1. Preliminary test of flavonoids*

The presence of flavonoids in the different extracts was demonstrated by the reaction to cyanidin (Shibata or Shinoda test) [16-18]. A few mg of the extract is dissolved in 50% of methanol. To this was added magnesium fragment then a few drops of concentrated hydrochloric acid (HCl). In fact, flavonoids are responsible for hydrogen evolution and the appearance of a colour ranging from orange yellow to purple red.

*2.6.2. Separation of the compounds by TLC*

For obtaining a flavonic footprints for our extracts, and know an idea on their chemical composition, an analytical thin-layer chromatography (TLC) with silica gel [19] was performed, in order to separate these compounds, using a solvent system (butanol, ethyl acetate, petroleum ether) and a solution of $FeCl_3$ (1%) for revelation.
The choice of mobile phase (appropriate solvent system) was done after testing several solvent mixtures. This giving the best separations were: butanol / ethyl acetate / petroleum ether (60/20/20) (V / V / V).
Visualization of spots was made under UV at 254 nm and 366 nm and by the disclosure in $FeCl_3$, and Rf (the ratio of the distance travelled by this molecule on the distance travelled by the mobile phase that is to say the solvent front) is measured.

*2.7. Antimicrobial activity evaluation*

*2.7.1. Microorganisms Strains*

The petroleum ether, ethyl acetate and n-butanol *Mentha pulegium L.* extracts were individually tested against four bacterial strains common in human pathology, belonging to Gram positive and Gram negative classes, (with the following bacteria) were: Pseudomonas aeruginosa ATCC27853 (Gram negative), Escherichia coli ATCC25922 (Gram negative), Klebsiella pneumoniae ATCC700603 (Gram negative) and Staphylococcus aureus ATCC25923 (Gram positive). These bacterial species are responsible for skin infections (Staphylococcus aureus), urinary and digestive tract infections (Escherichia coli) and nosocomial infections (Klebsiella pneumoniae and Pseudomonas aeruginosa).

*2.7.2. Biological assays*

Antimicrobial activity was performed with the Bauer-Kirby method by (by using the disk diffusion technique) [20] the well diffusion technique using Petri plates made by Muller Hinton agar containing the culture. This run involved the preparation of sterilized Whatman filter paper cut in form of disc of 0.4 cm of diameter, which was impregnated with the compound to be tested (500 mg/mL in DMSO), followed by adhering it to the surface of Petri plate that was previously inoculated with diluted culture. The incubation was realized at 37 °C for 24h. The results, expressed as the inhibition aureole diameters in millimetre (mm) and the MIC (mg/mL) (For this method, the MIC is defined as the lowest concentration of extract to which it may have an effect).
The antibacterial activity is determined in terms of diameter of the zone of inhibition produced around the disks after 24 hours of incubation at 37 ° C.
The percentage inhibition of fungal growth is calculated by the following formula:

$$\%Inhibition = \left( \frac{D_{test}}{D_{control}} \right) \times 100$$

$D_{test}$ : diameter of the inhibition zone.
$D_{control}$ : diameter of the Petri dish.

*2.8. Statistical Analysis*
All the samples were analyzed in triplicate, except those for EPR method which were analyzed in duplicate; the average and the relative SD were calculated using the Excel software package.

## 3. Results and discussion

*3.1. Extraction's results*
Each extract was characterized by the aspect, colour and yield reported for the dry matter. The results are presented are presented in Table 1 and 2. The water content found was 68.7 ± 5.11% for the month of February, 64% ± 4.2 for the month of March and 61.5% ± 3.10 for April.

**Table 1:** Characteristics of phenolic extracts obtained from the *Mentha pulegium L.* leaves.

| Extracts | Aspect | Colour |
|---|---|---|
| petroleum ether (PE) | oily paste | green |
| ethyl acetate (EtOAc) | pasty | dark green |
| n-butanol (BuOH ) | powder | brown |
| Aqueous (Aq) | powder | dark brown |

**Table 2:** Results of mass yields reported for the dry matter of *Mentha pulegium L.* leaves.

| | mass(g) | | | yield (%) / the plant mass | | | yield (%) / the crude extract | | |
|---|---|---|---|---|---|---|---|---|---|
| | Feb | Mar | Apr | Feb | Mar | Apr | Feb | Mar | Apr |
| Crude extract (ethanol) | 0.89 | 2.27 | 1.74 | 6.84 | 17.46 | 13.38 | 100 | 100 | 100 |
| Petroleum ether | 0.18 | 0.50 | 0.62 | 1.38 | 3.85 | 4.77 | 20.23 | 22.03 | 35.63 |
| Ethyl acetate | 0.19 | 0.10 | 0.21 | 1.46 | 0.77 | 1.62 | 21.35 | 4.41 | 12.07 |
| n-butanol | 0.14 | 0.21 | 0.20 | 1.08 | 1.62 | 1.54 | 15.73 | 9.25 | 11.49 |

The results are reflected in the diagrams of figure 2.

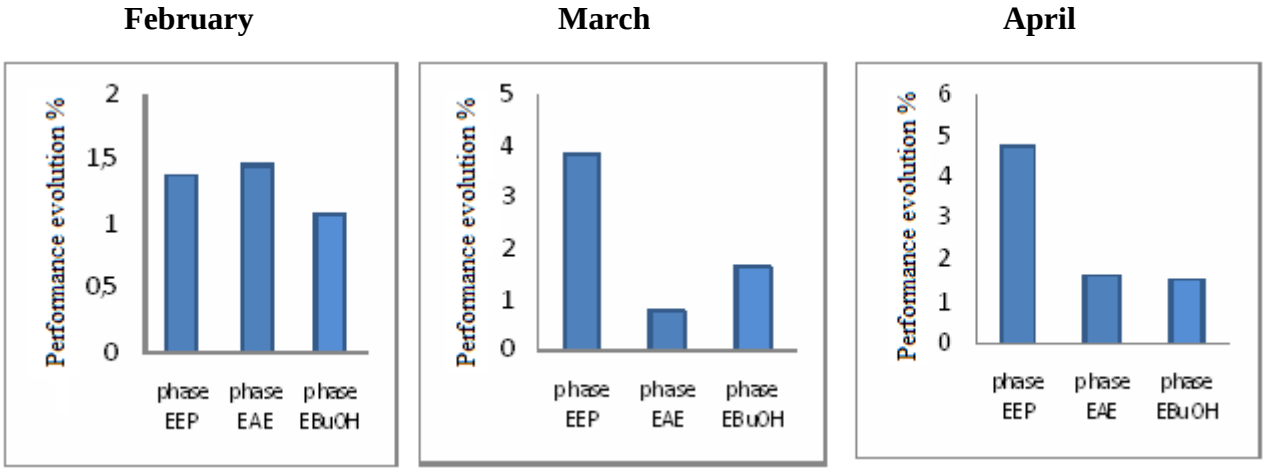


**Figure 2:** Performance evolution dry extract of dried leaves of *Mentha pulegium L.* as a function of the extraction solvent.

The results show a high yield of crude extract for the extraction performed by Soxhlet ethanol (17.46%) in March, and the least important is obtained with ethyl acetate (4.41%) in April. The performance of petroleum ether extracts are the highest (20.23%, 22.03% and 35.63%) and that of 21.35% ethyl acetate in February.
This difference can be explained by the fact that the petroleum ether and ethyl acetate are no polar organic solvents used to degrease drugs, usually extract the lyophilise compounds (lipids, fatty acids, arytenoids, chlorophylls ...) therefore our plant is rich in loopholes. However, these extractions can be regarded as complementary to the extent that the natural products have quite different polarities.

*3.2. Total Polyphenolic Contents (TPC)*

The total phenolic content of the *Mentha pulegium* extracts (in Gallic acid equivalents) are presented in Table 4. The concentrations of polyphones are calculated by interpolation on the calibration curve Figure 3.

**Table 3:** The evaluation of Gallic acid absorbance versus their concentrations**.**

| Concentration (µg/mL) | 5.75 | 10.5 | 23 | 45 | 100 |
|---|---|---|---|---|---|
| ABS | 0.111 | 0.204 | 0.312 | 0.587 | 1.137 |

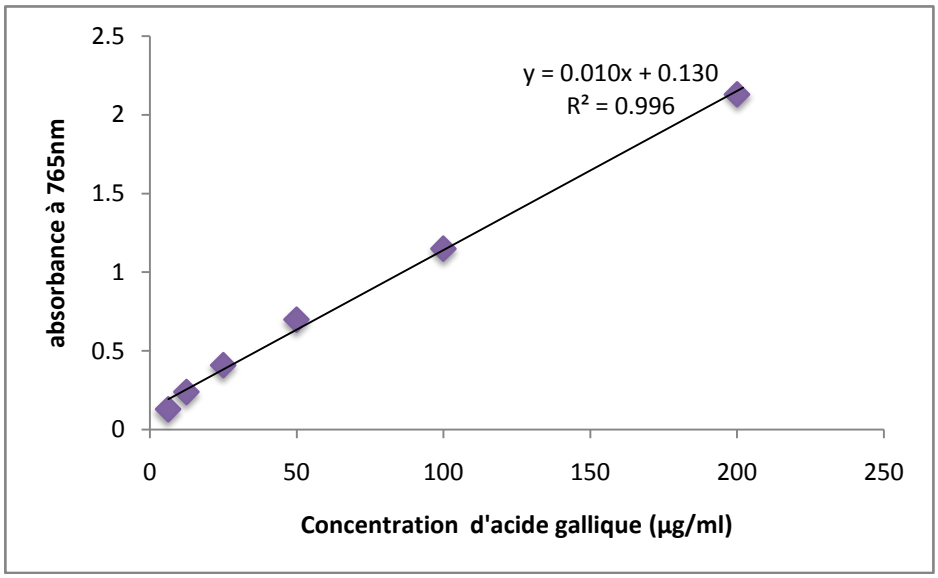


**Figure 3:** Calibration line of polyphenol (mean of three measurements).

**Table 4:** Contents of total polyphenols in *Mentha pulegium L.* leaves extracts

| Extract | Polyphenol content (µg EAG/mg)[a] | | |
|---|---|---|---|
| | February | March | April |
| petroleum ether (PEE) | 31.37 ± 2.2 | 53.18± 3.2 | 4.36±0.2 |
| ethyl acetate(EAE) | 115.81± 4.1 | 277.38± 4.12 | 61.36± 1.1 |
| n-butanol (BuE) | 82.63± 3.2 | 124.81± 3.13 | 32.16± 1.12 |
| Extract | Polyphenol content (µg EAG/mg)[a] | | |
| | February | March | April |
| petroleum ether (PEE) | 31.37 ± 2.2 | 53.18± 3.2 | 4.36±0.2 |
| ethyl acetate(EAE) | 115.81± 4.1 | 277.38± 4.12 | 61.36± 1.1 |
| n-butanol (BuE) | 82.63± 3.2 | 124.81± 3.13 | 32.16± 1.12 |

[a] *Total phenolic content is expressed as Gallic acid equivalents*
*The values (average of three replicates ± standard deviation)*

The choice to quantify polyphenols among different phytochemicals, results from the fact that polyphenols have very important biological activities [21]. The polyphenols concentrations are determined from the calibration straight lines ($y = 0.010x + 0.130$, $R^2 = 0.996$) plotted using Gallic acid as standard.

The obtained results presented in Fig. 3 showed that TPCs varied significantly as a function of period as well as the nature of extraction solvent. The highest values were recorded in the March ethyl acetate extracts (277.38±0,775 µg EAG/g DW). The lowest content of total phenols (32.16 ± 1.12 µg EAG/g DW) was observed in the april butanol extract. The phenolic content of a plant depends on a number of intrinsic factors (genetic) and extrinsic (climate, cultural practices, harvesting and storage conditions) [22].

*3.3. Identification of flavonoids and separation of phenolic compounds*
*3.3.1. Preliminary test of flavonoids (Shibata or Shinoda test)*
The flavonoids are considered the most important class of polyphenols. The colorations test of substances in different *Mentha pulegium L*. extracts gave positive reactions for the three months (February, March and April). The results of this study are reported in table 5.

**Table 5:** coloration test results

| Extracts | Obtained colour | Result of Shinoda test |
|---|---|---|
| Petroleum ether extract | Brown | - |
| ethyle acetate extract | Orange | + |
| Butanol extract | yellow | + |

Shibata test [22] showed that the *Mentha pulegium* is rich in Flavonoïdes. We noticed that each extract phase gave a different colour to another, orange to yellow, with variation in colour intensity.

*3.3.2. Separation of flavonoids by thin layer chromatography "TLC"*
After the revelation of the TLC plate with FeCl3 (1%), three main spots were observed for each month (Table 6). For the Petroleum ether extract the Rf values was (0.81, 0.84 and 0.86) for the three months (February, March, April) respectively, or these values are not identified; ethyle acetate extract was an Rf equal to (0.73, 0.75, 0.75), so this extract is rich in flavanones, flavonols or methoxyflavones; and for the BuOH extract their Rf are (0.35, 0.37, 0.39), these values can express the presence of the compounds and oligohydroxy compound oligomethoxyflavones.

**Table 6:** chromatographic behavior of *Mentha pulegium L.* extracts in the solvent system (butanol/ethyl acetate / petroleum ether) (8/2/2) (V / V / V)

| Extract | Rf | | | Interpretation according to [19] |
|---|---|---|---|---|
| | February | March | April | |
| Petroleum ether extract | 0.81 | 0.84 | 0.86 | Only contains fats, chlorophylls, and impurities. |
| Ethyle acetate extract | 0.74 | 0.75 | 0.75 | Rf (0.5-0.75)<br>Flavanones, flavonols, méthoxyflavones |
| BuOH extract | 0.35 | 0.37 | 0.39 | Rf (0.3-0.5)<br>The oligohydroxy compound and oligométhoxyflavones |

The amount of phenolic compounds in extracts of the plant depends mainly studied: their origin, variety, the growing season, the harvest season, climate and environmental conditions, the geographical location, the different diseases that can affect the plant, the maturity of the plant and the shelf life [24, 25].

*3.4. Antibacterial Activity*
The diffusion test was applied to four microorganisms including Gram-positive and Gram-negative bacteria. The antimicrobial capacity is obtained by measuring the diameters of zones of inhibition in millimetres. The results are summarized in Tables 7, 8 and 9 which shows that the extracts prevented the growth of all the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested extracts.
The scale for estimating the antimicrobial activity is given by Meena and Sethi [26], it has classified the diameters of inhibition zones (D) of the microbial growth in four classes: Highly inhibitory: $D \geq 28$ mm; inhibitory moderately: $16 \leq D < 28$ mm; inhibitory slightly $10 \leq D < 16$ mm; Not inhibitory: $D < 10$ mm.

**Table 7:** Values of the diameters of inhibition zones and the% inhibition extracts of *Mentha pulegium L*

| Microorganisms | | ***Diameter of inhibition (mm)*** | | | ***Inhibition percentage (I)*** | | |
|---|---|---|---|---|---|---|---|
| | | ***EEP*** | ***EAE*** | ***EBuOH*** | ***EEP*** | ***EAE*** | ***EBuOH*** |
| ***Gram +*** | *Staphylococcus aureus* | *20* | *16* | *16* | *26.67* | *21.33* | *21.33* |
| ***Gram -*** | *Klebsciella pneumoniae* | *21* | *18* | *23* | *28* | *24* | *30.67* |
| | *Escherichia coli* | *19* | *24* | *22* | *25.33* | *32* | *29.33* |
| | *Pseudomonas aeruginosa* | *11* | *08* | *09* | *14.67* | *10.67* | *12* |

The results show that the diameter of the inhibition zone differs from a bacterium to another and from a sample to else. The extracted petroleum ether, ethyl acetate and n-butanol for our plant are moderately active in the three bacterial strains: Staphylococcus aureus, Escherichia coli and Klebsciella pneumoniae (16 <D <28mm), excluding the Pseudomonas aeruginosa strain, it is non-inhibitory (D <10mm) for ethyl acetate, BuOH extracts, and resistant to the petroleum ether extract.

**Table 8:** Values diameters (mm) of inhibition zones for deferent concentrations of *Mentha pulegium L.* extracts.

| Microorganisms | | Extracts concentrations (mg/mL) | | | |
|---|---|---|---|---|---|
| | | 0.50 | 0.25 | 0.125 | 0.0625 |
| *Staphylococcus aureus ATCC25923* | PE | 20±1.72 | 18±0.62 | 17±0.5 | - |
| | EtOAc | 24±1.61 | 20±0.63 | 18±0.4 | - |
| | *n*-BuOH | 23±0.76 | 22±0.52 | 20±0.63 | - |
| *Klebsiella pneumonia ATCC700603* | PE | 28±1.77 | 20±0.62 | - | - |
| | EtOAc | 23±0.38 | 20±0.66 | 19±0.75 | - |
| | *n*-BuOH | 25±0.52 | 23±0.8 | 18±0.62 | - |
| *E. coli ATCC25922* | PE | 24±0.52 | 22±1.72 | - | - |
| | EtOAc | 20±1.32 | 16±0.52 | 15±0.62 | - |
| | *n*-BuOH | 19±2.56 | 16±1.5 | 15±0.51 | - |
| *Pseudomonas aeruginosa ATCC27853* | PE | 11±1.77 | 09±0.62 | 08±0.5 | - |
| | EtOAc | 08±0.76 | - | - | - |
| | *n*-BuOH | 09±1.04 | - | - | - |

Values are means ± SD of three separate experiments done in triplicate.
( - ): lecture impossible.

The decrease in the diameter of the inhibition zones corresponding to a decrease of the concentration of extract applied. The extracts diluted of *Mentha pulegium L.* (Table 9) exerted a significant inhibitory activity against the tested bacteria, in fact, all bacterial strains were inhibited at a concentration between 0.125 g/mL and 0.25 mg/mL, with the exception of P. aeruginosa, which describes a low resistance against the ethyl acetate and BuOH extracts, and the minimum inhibitory concentration of 0.50 g/mL against ethyl petroleum extract.

**Table 9:** Minimal inhibitory concentration (MIC) of *Mentha pulegium* L. against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae.*

| ***souches testées*** | | MIC **(mg/mL)** | | |
|---|---|---|---|---|
| | | ***EEP*** | ***EAE*** | ***EBuOH*** |
| ***Gram +*** | *Staphylococcus aureus* | *0.125* | *0.125* | *0.125* |
| ***Gram -*** | *Klebsiella pneumoniae* | *0.25* | *0.125* | *0.125* |
| | *Pseudomonas aeruginosa* | *0.50* | - | - |
| | *Escherichia coli* | *0.25* | *0.125* | *0.125* |

The optimal efficiency of an extract can not be due to a main active ingredient, but the combined action (synergy) of different compounds at the origin of this extract [27].

## Conclusion

The present work is a contribution to the study of extraction and biological activities of some secondary metabolites of the *Mentha pulegium* L. from the Tarik Ibn Ziad region (Ain-Defla, Algeria). The plant is subjected to Soxhlet extraction, and by solvents of different polarity, quantitative analysis by spectrophotometry and TLC revealed significant levels of polyphenols with significant amounts of flavonoids.

From the results of the present study, it appears that the tests of antibacterial activity of different extracts showed inhibition against Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli, and a resistance by Pseudomonas aeruginosa.

## References

1. Le Houerou H.N., Claudin J., Pouget M., Etude bioclimatique des steppes algeriennes, *Bull. Soc. Hist. nat. Afr. Nord Alger*, t. 68, fasc. 3 et 4, (1977)
2. Benabadji N., Bouazza M., *Rev. Energ. Ren*. 3 (2000) 117.
3. Boukhatem M.N., Saidi F., Hamaidi M.S., Hakim Y., Mekarnia M., *Phytother*. 9 (2011) 304.
4. de Fátima C., Branco Rangel de Almeida C., Lyra de Vasconcelos Cabral D., Castelo Branco Rangel de Almeida C., Lúcia Cavalcanti de Amorim E., Magali de Araújo J., Paulino de Albuquerque U., , *Pharm. Biol.* 50 (2012) 201.
5. Belmekki N., Bendimerad N., Seladji M., *J. Nat. Prod. Plant Resour*. 2 (2012) 558.
6. Reguieg L., *Am. J. Food. Nutr*. 1 (2011) 126.
7. Mamedov N., Gardner Z., Craker L.E., *J. Herbs Spices Med. Plants* 11 (2004) 191.
8. Mhamd B., Abbassi F., Abdell C., *Nat. Prod. Res.* 29 (2015) 1157.
9. Acquaviva R., Menichini F., Ragusa S., Genovese C., Amodeo A., Tundis R., R. Loizzo M., Iauk L., *Nat. Prod. Res.* 4-5 (2012) 475.
10. Kerbab K., Mekhelfi T., Zaiter L., Benayache S., Benayache F., Picerno P., Mencherini T., Sansone F., Aquino R.P., Rastrelli L., *Nat. Prod. Res.* 29 (2014) 671.
11. Tahri N., El Basti A., Zidane L., Rochdi A., Douira A., *Kastamonu Univ., Journal of Forestry Faculty* 12 (2012) 192.
12. Hmiri S., Amrani N., Rahouti M., *Acta Bot. Gallica* 158 (2011) 609.
13. El-Haci I.A., Atik-Bekkara F., *Ann. Biol. Res.* 2 (2011) 327.
14. Lee W.C., Peng C.C., Chang C.H., Huang S.H., Chyau C.C., *Molecules* 18 (2013) 1582.
15. Aboshora W.A., Lianfu Z., Dahir M., Qingran M., Qingrui S., Jing L., Nabil Qaid M Al-Haj N.QM., Ammar A., *Trop. J. Pharm. Res.* 13 (2014) 2057.
16. Jouahri D., Boudarene L., Sabaou N., *International Journal of Pharmacognosy and Phytochemical Research* 5 (2013) 45.
17. Aliyu A.B., Musa A.M., Abdullahi M.S., Ibrahi H., Oyewale A.O., *Acta Pol. Pharm.* 68 (2011) 67.
18. Taharaa S.A, Yasuyuki Hashidokoa & Junya Mizutani, *Agric. Bioi. Chem.* 51 (1987) 1039.
19. Bandyukova V.A., Shinkareako A.L., *Chem. Nat. Compd.* 9 (1973) 17.
20. Hachama K., Khodja M., Moulay S., Boutoumi H., Hennig L., Sicker D., *J. Heterocycl. Chem.* 50 (2013) 413.
21. Djeridane A., Yousfi M., Nadjemi B., Boutassouna D., Stocker P., Vidal N., *Food Chem.* 97 (2006) 654.
22. Gomez-Caravaca A.M., Gomez-Romero M., Arraez-Roman D., Segura-Carretero A., Fernandez-Gutierrez A., *J. Pharm. Biomed. Anal*. 41 (2006) 1220.
23. Yisa J., *Aust. J. Basic Appl. Sci.* 3 (2009) 3975.
24. Ebrahimzadeh M.A., Pourmmorad F., Hafezi S., *Turkish J. Biol*. 32 (2008) 43.
25. Park H.J., Cha H.C., *Korean journal of biological society*. 7 (2003) 327.
26. Hudaib M., Speroni E., Maria Di Pietra A., Cavrini V., *J. Pharm. Biomed. Anal.* 29 (2002) 691.
27. Essawi T., Srour M., *J. Ethnopharmacol*. 70 (2000) 343.