

Impact of pulsed electric field treatment for extracting essential oil from *Mentha Spicata L.*

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The impact of pulsed electric field (PEF) treatment on the extraction of essential oil from *Mentha spicata.L* (quantity and quality) has been studied. The high yield with a significant reduction in energy and extraction time were determined. The results showed that the maximum yield obtained by conventional hydrodistillation has 0.94% in 120 min of distillation. Almost the same yield of 0.90% was obtained after the application of PEF (2 kV/cm, 200 pulses) in only 60 min of distillation and a reduction of about 50% in energy used. Gas chromatography/mass spectrometry (GC/MS) analysis of *M. spicata* showed that oil quality was not affected by PEF treatment. The major components were present including carvone (31.07%; 33.09% with PEF), followed by limonene (13.84%, 15.26% with PEF), Eucalyptol (5.17%, 5.80% with PEF), Dihydrocarveol (1.74%, 2.11% with PEF), Caryophyllene (1.46%, 1.28% with PEF), Germacrene D (1.23%, 1.39% with PEF) and Fenchone (1.14%, 1.09% with PEF). The results showed appreciable antioxidant and anti-inflammatory powers of *M. spicata* essential oil.

Keywords: chemical composition, electric discharge, electroporation, essential oil, biological activity.

1. INTRODUCTION

Pulsed electric field (PEF) is an alternative treatment method, non-thermal and non-polluting. PEF is a treatment that involves the application of high voltage pulses electric for very short durations, on the order of microseconds to milliseconds, through a material placed between two electrodes [1-3] and which should be designed to minimize the effect of electrolysis. It is based on the mechanism of electroporation which increases the permeability of plant cells and allows the release of intracellular compounds from plant tissues [4-7].

PEF has useful applications in biotechnology, medicine, control of biofouling and sludge disintegration, and as a liquid food preservation technique [8], is used as a pre-treatment process on plant materials before conventional extraction to reduce the extraction power, used for food pasteurization, improving pressing and drying in the food industry [9, 10], moreover for intensification of essential oil yield and extraction of valuable components from different natural sources [11]. PEF can promote the coalescence of water droplets in water in oil emulsions [12]. This process does not affect the quality of the extracted products and improves the extraction rates and yields of different active ingredients [10].

Essential oils (EOs) are concentrated natural products with strong odors produced by aromatic plants as secondary metabolites, they have specific antibacterial powers [13], are of commercial importance and are used for pharmaceutical, agronomic, food, sanitary, cosmetic and perfumery purposes [14]. The quantity mined is relatively small with generally high prices [15].

Mints (*Mentha*) are perennial, herbaceous and very fragrant plants belonging to the *labiaceae* family. There are about 20 species, the most common of which are *watermint* (*Mentha aquatica*), *peppermint* (*Mentha piperita*), and *spearmint* (*Mentha spicata*) [16]. *Mentha* is used in traditional medicine to treat colds, fevers, and digestive and cardiovascular disorders. *Mentha* has many biological activities as antioxidant, antimicrobial, biopesticide, antitumor, anticancer, antiviral, antiallergic, anti-inflammatory and antihypertensive activity [17]. Mint EO is one of the most demanded substances in the essential oil trade worldwide [18]. Moreover, the method for obtaining the essential oil plays a key role and determines the quality of the oil [19].

For economic and environmental reasons, experiments have been made to improve the yield and quality of essential oil by using new technologies to reduce energy consumption and CO₂ emissions [19, 20]. This object of this work is to study the effect of PEF on the quality of the extracted oil, paying particular attention to improving the yield and reducing the processing time.

2. MATERIALS AND METHODS

2.1. Plants material

The samples of the aerial part (leaves + stems) *M. spicata* were collected in June (2021) in Mascara-Algeria (located in the north-west of Algeria at N35°26, E 02°11) then dried in the air and in the shade because light and temperature can cause chemical, physical and biochemical changes that will affect the quality of the oil [21].

2.2. PEF treatments

PEF treatment was carried out using a system designed and manufactured by the laboratory. This system offers exponential decay pulses with a high voltage $U = 6$ kV. The pulse frequency was kept constant at 1 Hz. The diagram of the PEF treatment system is shown in Figure 1 and is equipped with an adjustable high voltage power supply, energy storage capacitors ($4\mu\text{F} \pm 5\%$, 3000Vdc, 115A rms), a discharge switch and a chamber treatment consisting of two electrodes flat and parallel in stainless steel separated by a distance of 1.5 cm from each other.

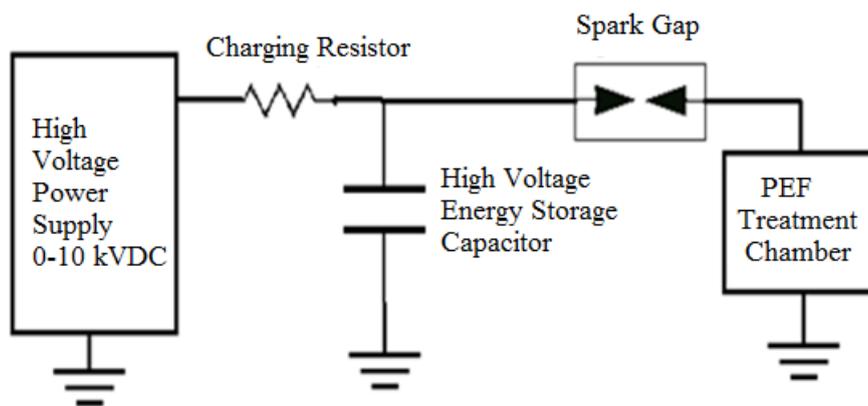


Figure 1. PEF treatment system

A 20 g sample with water placed between the electrodes is subjected to an electric field of intensity:

$$E = \frac{U}{d} \quad (1)$$

With, U: voltage, d: distance between electrodes. The ratio of water to material was 3:1 (mL:g). Then, the samples were subjected to PEF treatment under an electric field intensity of 1 kV/cm and 2 kV/cm with exponential decay pulses of 100 and 200 pulses. The temperature of the treatment medium was measured with a digital thermometer before and after the PEF treatment and the temperature variations were always less than 2°C. The effects of electric field intensity (1, and 2 kV/cm), number of pulses (100 and 200) and distillation time (30 and 60 min) on the yield of essential oil were examined.

2.3. Hydrodistillation

Extraction by hydrodistillation was carried out using a Clevenger-type apparatus standardized according to the European Pharmacopoeia. This process gives a good quality essential oil without any chemicals used. A mass of 20g of dried *Mint* was immersed in an extraction flask containing 200ml of distilled water. The whole is then brought to the boil using a balloon heater. The extraction times are 30, 60, 90 and 120 min for the control samples and 30 min then 60 min for the samples treated with PEF. The vapors are condensed in a cooler and collected in the Clevenger collector where the essential oil and water separate by difference in density. The essential oil was stored at 4°C in the dark before performing GC/MS analyses. The yield experiments for each parameter and each method were repeated at least 04 times, and the mean values were reported. The extraction yield of the essential oil *Y* was defined as the ratio between the mass *m* of the extracted essential oil and the total mass *M* of the treated plant material as follows [11]:

$$Y(\%) = \frac{m}{M} \cdot 100 \quad (2)$$

2.4. Chemical analysis

The GC/MS (Gas chromatography-mass spectrometry) analyzes were carried out on a Shimadzu capillary gas chromatograph directly coupled to the mass spectrometer system type GC-2010 plus, GCMS-TQ8030 from Shimadzu. A capillary column (30 mx 0,25 mm, 0,25 μm film thickness) was used under the following conditions: Column Oven Temperature: 50,0°C ; Injection Temperature: 230 °C ; Injection Mode: Split ; Pressure: 11.4 kPa ; Total Flow: 61.4 mL/min ; Column Flow: 1.42 mL/min ; Linear Velocity: 43,3 cm/sec ; Purge Flow: 3,0 mL/min and Split Ratio: 40.0. The column temperature was programmed from 50.0°C to 220.0°C with a rate of 5.00°C/min. The mass spectrometer (MS) conditions were as follows: Ion Source Temperature: 200.00 °C; Interface Temperature: 230.00 °C; Solvent Cut Time: 3,00 min and Detector Gain : 0.80 kV. The scanning mass range was 20-600 m/z, the total running time was 32 minutes (Scan Start : 45.00 m/z and Scan End : 600.00 m/z) and Scan Speed : 2000. Authentic chemicals are identified by the database NIST Chemistry WebBook 2021.

3. RESULTS AND DISCUSSION

3.1. Oil yield with conventional method

The kinetics is the monitoring of the evolution of the quantity of essential oil (EO) extracted by hydrodistillation of *M. spicata* as a function of time and without PEF treatment has been illustrated in Figure 2.

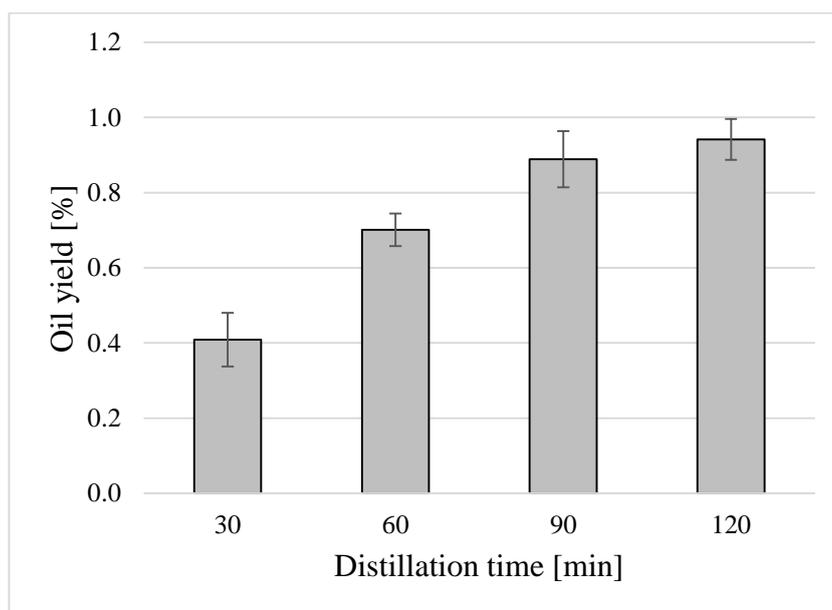


Figure 2. Yield of EO of *Mentha spicata* without PEF treatment

The average yields of essential oils were calculated based on the dry plant matter of the aerial part of the plant. The extraction yields were: $0.41 \pm 0.07\%$ (30 min); $0.70 \pm 0.04\%$ (60 min); $0.89 \pm 0.07\%$ (90 min) and $0.94 \pm 0.05\%$ (120 min). According to our experiments, we notice that the yield of the

essential oil increases with the distillation time and after 120 min of distillation, the yield of essential oil has reached its maximum value of 0.94%. Other work has already been done on the essential oil yields of *M. spicata* grown in Algeria with yields between 0.9% and 1.3% [22, 23]. Essential oil yields of *M. spicata* obtained by other authors in other countries were 0.53% [14] and 0.3% [24]. Certain ecological factors, the part of the plant used and the period of the vegetative cycle can be responsible for variations in the yields of EO of *M. spicata* [25].

In our work, we observed a significant increase between 30 min and 60 min (70.73%), between 30 min and 90 min (117.07%) and between 30 min and 120 min (129.26%). In addition, we found a significant increase between 60 min and 90 min (27.14%) and between 60 min and 120 min (34.28%). Finally, we noticed a non-significant increase of 5.62% when the distillation time goes from 90 min to 120 min. According to these results presented, we note that all of the oil was extracted between 90 min and 120 min of distillation and this is the maximum distillation time interval to extract the essential oil of *Mentha spicata* by the conventional method.

3.2. Effect of PEF on oil yield

In order to study the effect of pulsed electric field (PEF) treatment on the extraction yield of *Mentha spicata* EO, the samples are treated at different PEF parameters (1 kV/cm and 2 kV/cm for field strength (E) with 100 and 200 pulses), then extracted under the same conditions as a reference sample. Since the extraction protocol is identical to the reference one, any improvement in oil yield will be due to the application of PEF as a pre-treatment before extraction.

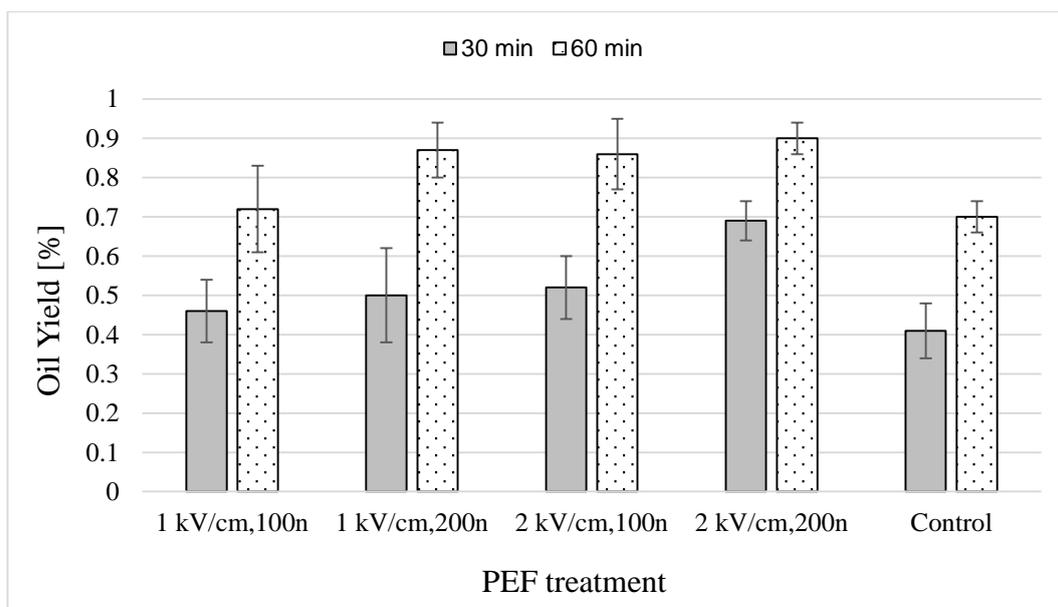


Figure 3. Yield of essential oil of *Mentha spicata* with PEF treatment

Longer distillation times lead to high temperatures which can cause chemical changes in the extracted EO and loss of the most volatile molecules [1, 26]. From an economic point of view, the PEF treatment technique has allowed us to have a quality essential oil with a shorter extraction time and for this reason we have chosen the distillation time of the samples treated with PEF at 60 min. In general, we observed that the EO yield of *M. spicata* was increased by the application of pulsed electric fields as shown in Figure 3.

This increase can then be explained with the modification of the content of the intercellular channels, the alteration of the structure of the cell membranes and the increase in the internal porosity induced in the plant matrix [27].

The results presented in Figure 3 show the EO yields obtained by applying PEF with an intensity of 1 kV/cm. Yields were $0.46 \pm 0.08\%$ (100 pulses) and $0.72 \pm 0.11\%$ (100 pulses) for distillation times of 30 min and 60 min respectively. Thus, a greater increase in essential oil was obtained for the same intensity and double the pulses (200n). After 30 min of distillation, the yield was $0.50 \pm 0.12\%$ and after 60 min of distillation the yield obtained was $0.87 \pm 0.06\%$.

The experimental results indicate an increase in extraction yield with the increase in the number of pulses from 100 to 200 pulses on the one hand and on the other hand with the increase in the distillation time from 30 to 60 min in agreement with [19, 28]. There, the essential oil yield for *M.spicata* treated by PEF of 1 kV/cm, increased from 12.20% for 100 pulses to 21.65% for 200 pulses compared to the untreated sample (Control) for a distillation time of 30min. For a distillation time of 60 min, the yield goes from 2.86% for 100 pulses to 24.26% for 200 pulses compared to the sample (Control). These results indicate that an intensity of 1 kV/cm was sufficient for permanent pores to be generated at the plant cell membrane, which enhances mass transfer and improves oil extraction [1]. To evaluate the efficiency of PEF for the extraction of *M.spicata* essential oil, we doubled the intensity of PEF (2 kV/cm) and performed the extraction with the same protocol and under the same conditions as a sample treated with PEF at 1 kV/cm and the reference sample.

The yields were: $0.52 \pm 0.08\%$ and $0.86 \pm 0.09\%$ with PEF (2 kV/cm, 100n) for distillation times of 30 min and 60 min respectively, then $0.69 \pm 0.05\%$ and $0.90 \pm 0.04\%$ with PEF (2 kV/cm, 200n) for distillation times of 30 min and 60 min respectively.

Depending on the PEF intensity of 2 kV/cm, the essential oil yield increased from 26.83% (100n) up to 68.29% (200n) compared to the control sample for the same distillation time of 30 min. as shown in figure 3. Thus after 60 min of distillation, the samples treated with PEF 2 kV/cm showed a higher yield compared to the control sample. An evolution of yields from 22.86% (100n) to 28.57% (200n) was achieved. After a distillation of 30 min and for samples treated with PEF (2 kV/cm, 200n), we observed a yield of 0.69% which is approximately the same yield obtained after 60 min of distillation (0.7%) for control samples.

By increasing the extraction time from 60 min to 120 min for the samples treated with PEF (2 kV/cm, 200n), the amount of oil increased slightly by 5.55%. The experimental data establish that for a short distillation time requires a high PEF value and for a medium distillation time the PEF values should be moderate. The results of this work are in agreement with the literature [28]. A non-significant evolution (1%) of the yield was reached between 60 min and 120 min for a sample treated with PEF (2V/cm, 200n) as indicated in Figure 4.

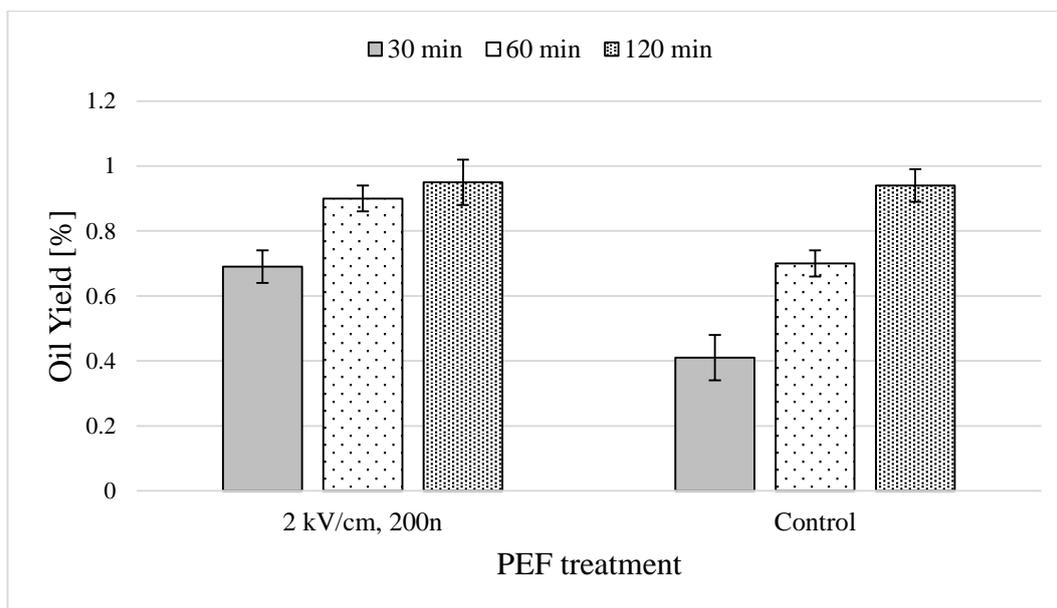


Figure 4 Comparison of optimal yields by conventional method and by PEF treatment

These results shown in figure 4, we allows to conclude that the parameters of treatment by PEF and extraction of the EO of *Mentha spicata* are: 2 kV/cm, 200 pulses and 60 min of distillation time. PEF treatment accelerates the kinetics of *M. spicata* leaf extraction. This is in agreement with the behavior observed in different fruit and vegetable tissues [7].

3.3. Chemical analysis

Analysis by gas chromatography/mass spectrometry (GC/MS) of the EO of *M. spicata* from a control sample (without PEF treatment) showed that carvone (31.07%) is the component major in agreement with other researchers [29]. The other main components of *M. spicata* oil obtained are limonene (13.84 %), Eucalyptol (5.17 %), Dihydrocarveol (1.74%), Caryophyllene (1.46 %), Fenchone (1.14 %), Germacrene D (1.23 %), trans-Carveol (0.92 %), β -Bourbonene (0.98 %) as shown in Table 1.

Our results are in agreement with previous studies, in which the predominant components of *M. spicata* oil were carvone (56.94 % [29] , 29 % [14] ,49.5 % [30], 76.65% [31]), suivie du limonène (11.63 % [29], 16.1 % [30], 9.57% [31]), germacrène D (2.1% [30]), dihydrocarvone (3.9% [30]), caryophyllène (2.7% [30]). Differences may be due to environmental conditions, crop conditions or [29] the nature of the soil.

After PEF treatment, the majority of the components obtained by the two methods were similar. Our results indicate an increase in certain main components including carvone (33.09 %), limonène (15.26 %), Eucalyptol (5.80 %), Dihydrocarveol (2.11 %), Germacrene D (1.39 %), trans-Carveol (1.14 %), β -Bourbonene (1.60 %) , also the decrease of some main components like Caryophyllene (1.28 %) et Fenchone (1.09 %) .

The application of PEF allows the appearance of certain components which are not in the control sample and on the other hand the disappearance of certain components already found in the control sample. This phenomenon is mainly due to the application of PEF which increased the permeability of the intracellular material and the cell disintegration index which facilitated the release of certain volatile components on the one hand and the transformation of certain components on the other part as it has been observed by several authors[2, 7, 31].

Table 1. Major Chemical Compounds

| Chemical Compounds* | Molecular Formula | Control** | After treatment PEF*** |
|--------------------------|--|-----------|------------------------|
| Dihydrocarveol | C ₁₀ H ₁₈ O | 1.74 | 2.11 |
| L-4-terpineneol | C ₁₀ H ₁₈ O | 0.69 | 0.78 |
| β-Pinene | C ₁₀ H ₁₆ | 0.77 | 0.93 |
| Sabinene | C ₁₀ H ₁₆ | 0.57 | 0.51 |
| Myrcene | C ₁₀ H ₁₆ | 0.49 | 0.51 |
| Jasmone | C ₁₁ H ₁₆ O | 0.38 | 0.50 |
| Limonene | C ₁₀ H ₁₆ | 13.84 | 15.26 |
| Eucalyptol | C ₁₀ H ₁₈ O | 5.17 | 5.80 |
| Caryophyllene | C ₁₅ H ₂₄ | 1.46 | 1.28 |
| Fenchone | C ₁₀ H ₁₆ O | 1.14 | 1.09 |
| 4-Thujanol, Stereoisomer | C ₁₀ H ₁₈ O | 0.83 | 0.96 |
| Germacrene D | C ₁₅ H ₂₄ | 1.23 | 1.39 |
| Pulegone | C ₁₀ H ₁₆ O | 0.41 | 0.47 |
| Myrtenyl acetate | C ₁₂ H ₁₈ O ₂ | 0.42 | 0.39 |
| Carvone | C ₁₀ H ₁₄ O | 31.07 | 33.09 |
| trans-Carveol | C ₁₀ H ₁₆ O | 0.92 | 1.14 |
| Caryophyllene epoxide | C ₁₅ H ₂₄ O | 0.42 | 0.43 |
| β-Bourbonene | C ₁₅ H ₂₄ | 0.98 | 1.60 |
| Camphor | C ₁₀ H ₁₆ O | 0.61 | - |
| (Z)-dihydrocarvone | C ₁₀ H ₁₆ O | 0.49 | - |
| cis-Dihydrocarvone | C ₁₀ H ₁₆ O | - | 0.51 |

*Area > 0.4%

**60 min of distillation

***2 kV/cm; 200 pulses; 60 min of distillation

(-) absence of a compound

3.4. Antioxidant activity

The antioxidant activity of *M. spicata* essential oils were evaluated by the phosphomolybdenum method according to the procedure describe by [32]. The assay was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex. Total antioxidant capacity of *M. spicata* extracts, expressed as mg/ml ascorbic acid equivalents (AAE), is shown in Figure 5.

Both essential oils (conventional methods and PEF) exhibited antioxidant effect while the highest antioxidant activity was found in conventional methods (CONV) essential oil with a value of 9.8 ± 0 mg/mL, followed by PEF (8.28 ± 0.05 mg/mL). Our results corroborate those of [33] who reported that the extracted EO was effective in reducing Mo (VI) to Mo (V) (RP50 value of 53.3 ± 2.8 $\mu\text{g/mL}$) increasing with the high concentrations.

In addition, *M. spicata* essential oil showed a reducing ability using other method such as ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) ($\text{IC}_{50} = 195.1 \pm 4.2$) and DPPH ($\text{IC}_{50} = 3476.3$ $\mu\text{g/mL}$) [34]. Similar results were reported by [23] on EO of Algerian mint (*M. spicata*) demonstrating a significant antioxidant activity ($\text{IC}_{50} = 10620$ $\mu\text{g/mL}$) which correlated to the high total phenolics content. These findings were supported by [35] who confirmed that *M. spicata* had the highest total phenolic content among the mint species grown in Iran.

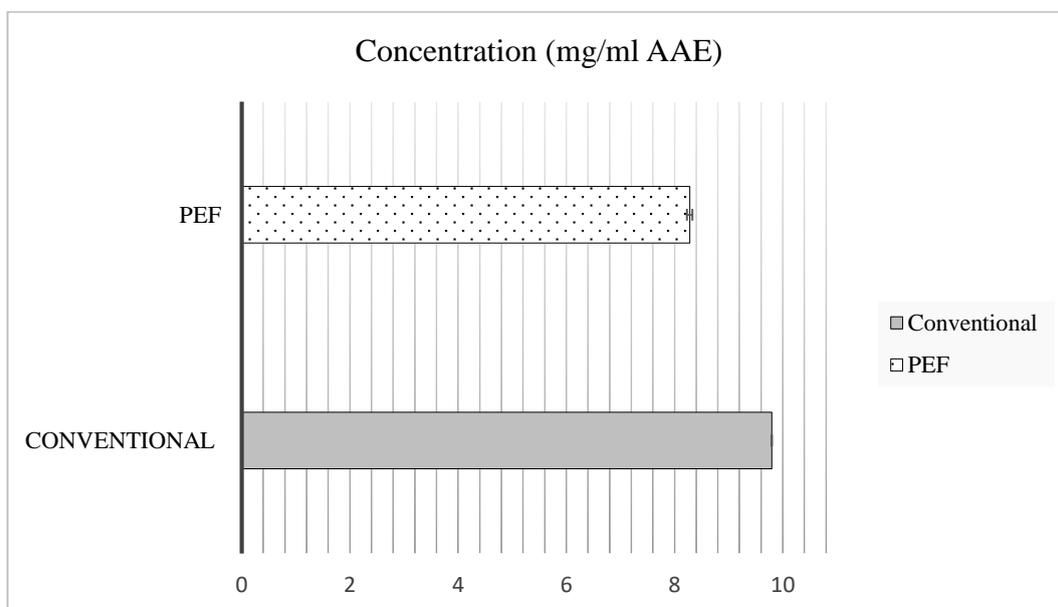


Figure 5. Total antioxidant capacity (TAC) of *M. spicata* essential oils Conventional method (60 min of distillation) and PEF (2 kV/cm, 200 pulses, 60 min of distillation)

3.5. Anti-inflammatory activity

Regarding the anti-inflammatory activity of *M. spicata* EO, the HRBC method (Human Red Blood Cell membrane) stabilization results are illustrated in Figure 6. Both *M. spicata* EOs exhibited a non-dose dependent HRBC membrane stabilizing activity. The stabilization activity exerted by *M. spicata* PEF EO was higher than that of CONV EO and sodium diclofenac. Indeed, at 500 and 1000 $\mu\text{g/mL}$, the HRBC stabilization activity of the PEF EO was found to be 25.145 ± 0.03 and $24.72 \pm 0.14\%$, respectively.

Whereas, at the concentration of 250 $\mu\text{g/mL}$, the CONV EO showed the highest HRBC stabilization activity of $19.98 \pm 0.16\%$. Our results are in agreement with those previously reported by Arumugam et al [36] who reported that *M. spicata* leaves had a potential anti-inflammatory activity. The

in vivo investigation on acute and chronic inflammation induced in rats revealed that both ethyl acetate and aqueous fractions of *M. spicata* were found to be effective in chronic inflammation; whereas, the ethyl acetate fraction was effective in acute inflammation.

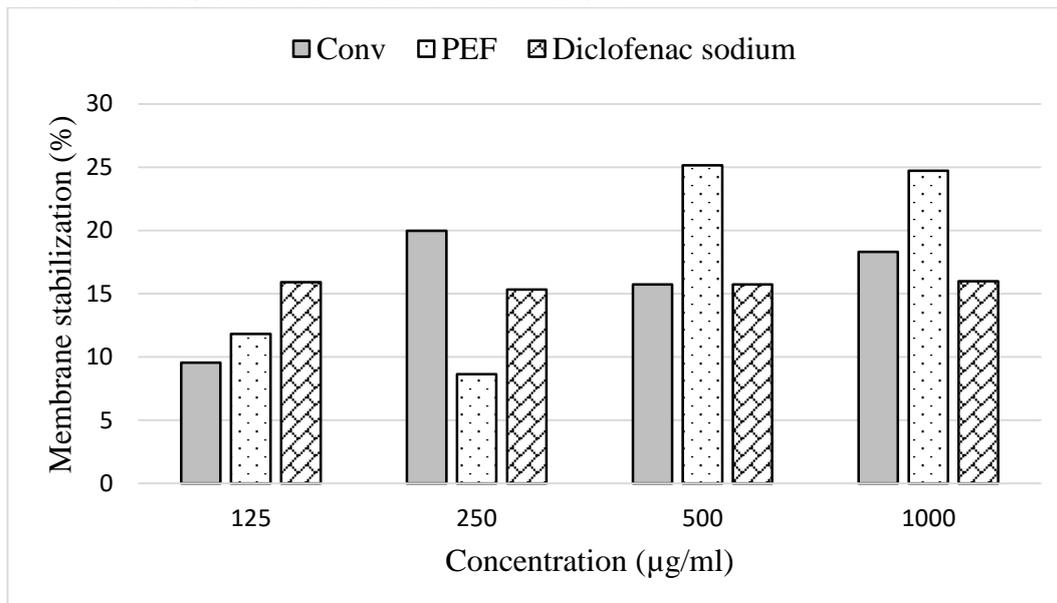


Figure 6. Anti-inflammatory activity of *M. spicata*

Conventional method (60 min of distillation) and PEF (2 kV/cm, 200 pulses, 60 min of distillation)

4. CONCLUSION

In the present study, the experimental results showed that the yield of the essential oil of *Mentha spicata* increased after the application of the PEF according to the three parameters: the intensity of the field, the number of pulses and the duration of distillation. The yield obtained by the conventional method (without PEF treatment) was 0.94% in 120 min of distillation, whereas by applying PEF we obtained almost the same quantity of oil (0.90%) in only 60 min, half the distillation time by the conventional method for the same operating conditions. PEF treatment could be proposed as an alternative method to intensify the extraction of essential oils in industry, especially for low-yielding plants.

GC-MS analysis indicated that the quality of the essential oil obtained after the PEF treatment did not degrade since the chemical composition was almost the same as that obtained with the conventional method. *M.spicata L.* essential oil has appreciable antioxidant and anti-inflammatory properties, which allows this plant to be considered as potential additives in food and pharmaceutical products.

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CONFLICT OF INTEREST

The authors declare that they have no conflict interests with the manuscript.

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