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EFFECT OF PULSED ELECTRIC FIELD TREATMENT ON THE EXTRACTION OF ESSENTIAL OIL FROM *LAVENDER (LAVANDULA ANGUSTIFOLIA* MILL.)

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ABSTRACT

This study aimed to analyze the effect of the pulsed electric field (PEF) on the increase of the extraction yield of essential oil from *Lavender* (*Lavandula angustifolia* Mill.*)*. PEF was applied to improve the permeabilization by electroporating the biological membranes, and the extraction of essential oil was performed using the hydrodistillation method. The influence of the voltage level, pulse number, and distillation process duration was studied. The results revealed that the extraction process was significantly improved when the proposed method was used as the amount of PEF-treated essential oil increased. In addition, PEF pretreatment causes a significant decrease in the distillation time. Moreover, physicochemical analysis of the essential oil under stress conditions shows us more stability.

Keywords: Pulsed electric field; Extraction; Essential oil; Aromatic plants; *Lavandula angustifolia*.

INTRODUCTION

Essential oils (Eos) are considered valuable raw materials in various industries (Balandrin *et al*., 1985), such as the cosmetic, soap, and fragrance industries which represent nearly 60% of the total demand for natural substances (Shahi *et al*., 2009). These industries are characterized by a wide variety of products with relatively small quantities and often high prices (Chauhan *et al*., 2009).

The name of the genus Lavandula comes from the Latin word "lavare", which means "to bathe" or "to wash", as it was used to wash oneself by the ancient Arabs, Greeks, and Romans (Pokajewicz *et al*., 2022). *Lavandula angustifolia* Mill. belongs to the family Lamiaceae. It is an herbaceous perennial plant widely distributed in moderate and subtropical regions. *Lavender is* an indigenous plant to Africa, the Arabian Peninsula, the Mediterranean basin and Russia (Wilson *et al*., 2021; Kiran Babu *et al*., 2016). It is commonly known as 'English Lavender' or 'True Lavender'. Its essential oil (EO) is a very fragrant, refreshing, sweet and balsamic herbaceous smell giving a clean feel and woody undertones (Kiran Babu *et al*., 2016). In agri-food manufacturing, lavender essential oil is used to flavor pastries, ice creams, drinks, sweets and chewing gum (Smigielski *et al*., 2009).

In modern times, lavender is cultivated worldwide. The fragrant oils extracted from their flowers are used in baking, tea, jellies, candles, detergents, perfumes, cosmetics, shampoo, soap, massage oils, powders as well as in aromatherapy. The infusion of the aerial parts of *Lavandula* species is used as an appetite stimulant as well as in traditional medicine for the cure of bronchitis, asthma, colds, colic and fatigue (Jianu *et al*., 2013). *Lavandula angustifolia* oil is highly active against many species of viruses, fungi, and bacteria. The EO may be very indicated to treat bacterial infections resistant to antibiotics (Smigielski *et al*., 2009).

EO can usually be extracted from the plant using different methods, such as vacuum distillation, steam distillation, supercritical CO_2 extraction and solvent extraction. The oil content composition may vary depending on the place of origin, the harvest season and the environmental conditions. The method of obtaining the EO plays a key role in determining the quality of the oil (Yajun *et al*., 2017).

For economic and environmental reasons, the agri-food and chemical industries are facing a challenge as they have to use new technologies to reduce CO_2 emissions and energy consumption (Bousbia *et al*., 2009). Several unconventional methods have been investigated to improve the overall yield and selectivity of bioactive components from plant material. We can mention the microwave heating (Kaufmann and Christen, 2002), ultrasound (Ghafoor *et al*., 2011), pulsed electric field (PEF) (Toepfl *et al*., 2006), supercritical fluids (Marr and Gamse, 2000) and ohmic heating (Lakkakula *et al*., 2004). Solvent extraction can improve the oil yield, but it is not easy to obtain solvent-free products. The application of the supercritical CO_2 extraction method is limited because of its high cost (Yajun *et al*., 2017) .

The extraction yield from different plants such as *Rosa damascene* (Tintchev *et al*., 2012), *Eucalyptus, Thyme* (Barros *et al*., 2022) and Marrubium (Miloudi *et al*., 2018) can be improved considerably using PEF. The mechanism of extraction is based on the electroporation of the cell membranes using high-level electrical field pulses. Thus, the intracellular content is released more easily. Due to the application of PEF, the intracellular content is released and therefore it facilitates the processes of diffusion in the tissues and the mass exchange with the external environment. Thus, the technique minimizes environmental risks by emitting less CO_2 into the atmosphere(Ferhat *et al*., 2006) . Using PEF-assisted extraction techniques is an interesting alternative and more effective method than other conventional extraction processes.

The main objective of this work is to analyze the effect of PEF pretreatment on the yield, anti-inflamatory activity and chemical composition of *Lavandula angustifolia*.

MATERIALS AND METHODS

Plant material

Lavandula angustifolia Mill. used in our study is purchased as a dry flower from an herbal seller in the Mascara area, located in northwest Algeria at 35° 23' 25" N latitude and 0° 8' 58" E longitude. The Mascara region is located in the semi-arid climate zone. It is characterized by two main periods: a dry and hot period during the months of May to September (in which the absolute maximum air temperature is about +42°C) and another rainy and dark period during the months of November to April (in which the absolute minimum air temperature is equal to –4 ° C) (Laounia *et al*., 2017).

PEF treatments and essential oil extraction

The PEF equipment and protocol of essential oil extraction used in this work were previously described by (Miloudi *et al*., 2018). The extraction by hydrodistillation was performed by using a Clevenger-type apparatus standardized according to the European Pharmacopoeia.

Several distillations were carried out by boiling 60 g of plant material with water. The yield experiments for each parameter and each method were repeated at least 04 times, and the mean values were reported. Before analysis by gas chromatography-mass spectrometry (GC/MS), the essential oil was stored in the dark at temperature of 4°C.

The essential oil extraction yield is defined as the ratio between the mass (m) of the essential oil, obtained by extraction, and the total mass (M) of the treated plant material, as follows:

$$Y\ (\%) = (m / M) \times 100 \quad (1)$$

The sample of 60 g with water placed between the electrodes is subjected to an electric field of intensity E=U/d (U: voltage, d: distance between electrodes). The ratio of water to the material was 3:1 (mL:g). The used treatment chamber consists of two parallel stainless steel electrodes separated by d=1.5 cm, which represents the sample thickness.

PEF treatment system (Fig.1) includes a DC high-voltage source (U= 10 kV), a treatment chamber, a spark gap switch, and an energy storage capacitor. The high-voltage supply charges all the capacitors until the spark gap breakdowns, thus causing an abrupt voltage (shock) to be applied to the load (treatment chamber where the sample is disposed).

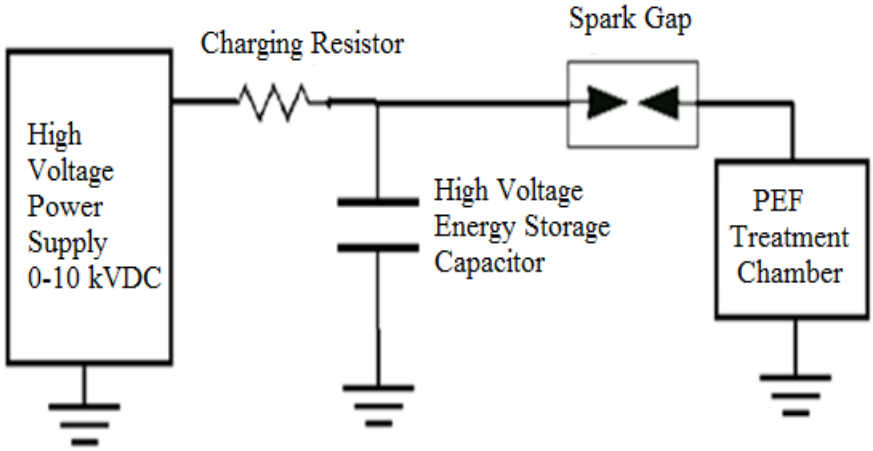

Fig. 1. PEF treatment system.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC analysis of volatile samples produced by different extraction techniques was performed on the Shimadzu GC-2010 plus gas chromatograph, GCMS-TQ8030 from Shimadzu (Tokyo, Japan), equipped with a flame ionization detector (FID) and BP-20 (SGE International, Ringwood, Australia) capillary column, 30 m length × 0.25 mm internal diameter, 0.25 µm film thickness (polyethylene glycol, TPA-treated), oven temperature programmed as follows: Column Oven Temperature: 50,0°C; Injection Mode: Split; Pressure: 11.4 kPa; Injection Temperature: 230 °C; Column Flow: 1.42 mL/min ;Total Flow: 61.4 mL/min ; Purge Flow: 3.0 mL/min; Linear Velocity: 43.3 cm/sec and Split Ratio: 40. The column temperature was programmed from 50°C to 220°C with a rate of 5°C/min. The mass spectrometer (MS) conditions were as follows: Ion Source Temperature: 200°C; Interface Temperature: 230 °C; Solvent Cut Time: 3 min and Detector Gain: 0.8 kV. The scanning mass range was 20-600 m/z, the total running time was 32 minutes (Scan Start: 45 m/z and Scan End: 600 m/z) and Scan Speed: 2000. Authentic chemicals were identified by the database NIST Chemistry WebBook 2021.

pH measurement

It is envisaged to compare the stability of pH of conventional essential oil and the treated one as a function of the various temperatures. Therefore, the pH values of both essential oils were measured by immersion of the pH electrode into the sample of 0.2mL using a pH meter (Orion Star A111, USA). Each measurement was taken after 5 minutes of temperature maintenance.

Determination of anti-inflammatory activity

The anti-inflammatory activity, a human red blood cell HRBC membrane stabilizing assay was performed as described in Sunmathi *et al*. (2016). 1 mL of various concentrations of the extracts (125, 250, 500, 1000µg/mL) was added to 1 mL of phosphate buffer, 2 mL hypo saline (0.25% w/v NaCl) and 0.5 mL of HRBC suspension. The reaction mixture was incubated at 37°C for 30 min then centrifuged at 300 rpm for 20 min. The sodium diclofenac has been used as a reference medication for this assay. The hemoglobin content of the supernatant solution was spectrophotometrically estimated at 560 nm as:

$$\text{Membrane stabilization (\%)} = 100 - (A1\text{-}A2/A1\times100) \quad (2)$$

Where A1 is the absorbance of the hypotonic-buffered saline solution alone and A2 is the absorbance of a test sample.

RESULTS AND DISCUSSION

Oil yield without PEF treatment

In the present study, we have shown in Fig.2 the extraction yields of the EO from *Lavandula angustifolia* at different extraction times. The results were respectively 0.799% in 15 min of distillation, 2.331% in 30 min, 2.820% in 45 min, and 2.953% in 60 min and the maximum yield obtained in our study was 3.150% in 120 min of distillation. Some work carried out previously indicated yields 2.13% (Slimani *et al*., 2022), 1.5% (Elharas *et al*., 2013) and 2.34% (Verma *et al*., 2010).

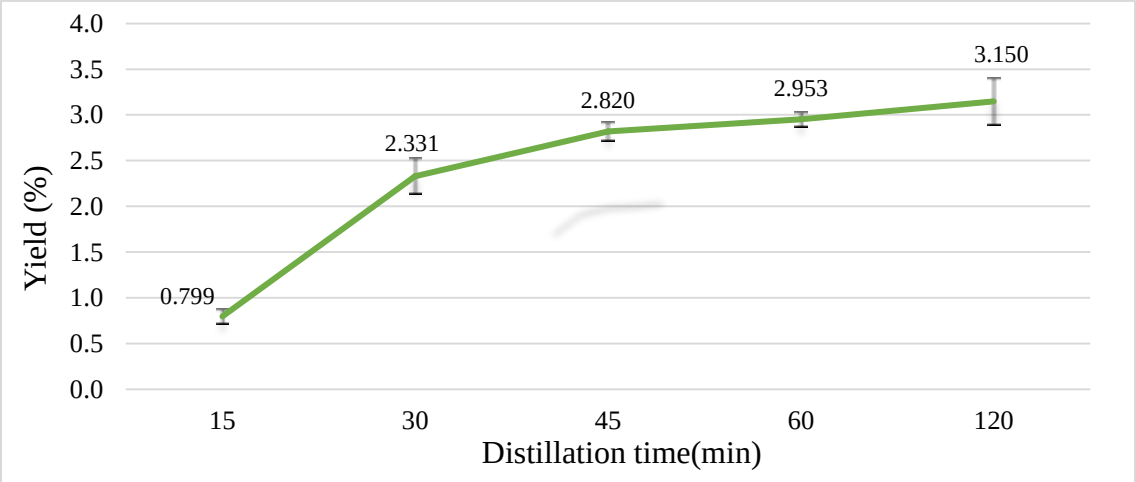

Fig.2. Yield of essential oil from *Lavandula angustifolia* without PEF treatment

The change in geographical location causes this difference in the quantity of oils (altitude and latitude), the variation in the nature of the soil, the harvest season and the climatic conditions (Bowles, 2020). We have shown that the extraction yields will be the highest for longer distillation times. Indeed, we observed that when the distillation time increases from 15 min to 30 min, the rate of increase in the oil distillation yield is 191.74%. Then, increasing the extraction process by 15 min (from 30 min to 45 min) increased the yield by 20.98%. In addition, a rate of increase of 4.72% was observed between 45min and 01h. When we increased the distillation time to 2h (01h more distillation), we noticed a non-significant increase of 6.67%. From these results, it can be said that the minimum distillation time from *Lavandula angustifolia* is 30 min and the optimum is 01 h distillation by conventional extraction.

Oil yield after PEF treatment

Applying a PEF treatment before the extraction of *Lavandula angustifolia* by hydrodistillation generally and significantly increased the yield of EO, as shown in Figures 3 and 4. The best oil yields were obtained using an electric field intensity of 1 kV/cm (Fig.3).

In just 15 min, the quantity of oil obtained by PEF (1 kV/cm, 100 pulses) increased by 18.96% compared to the reference (30 min of distillation). In addition, the amount of oil extracted with PEF (1kV/cm, 100 pulses) and PEF (1 kV/cm, 200 pulses) was much greater in 30 min of distillation with yields of 3.046% and 3.075 %, respectively, than the quantity of oil obtained in 60 min by the conventional method (Control) which was 2.953%.

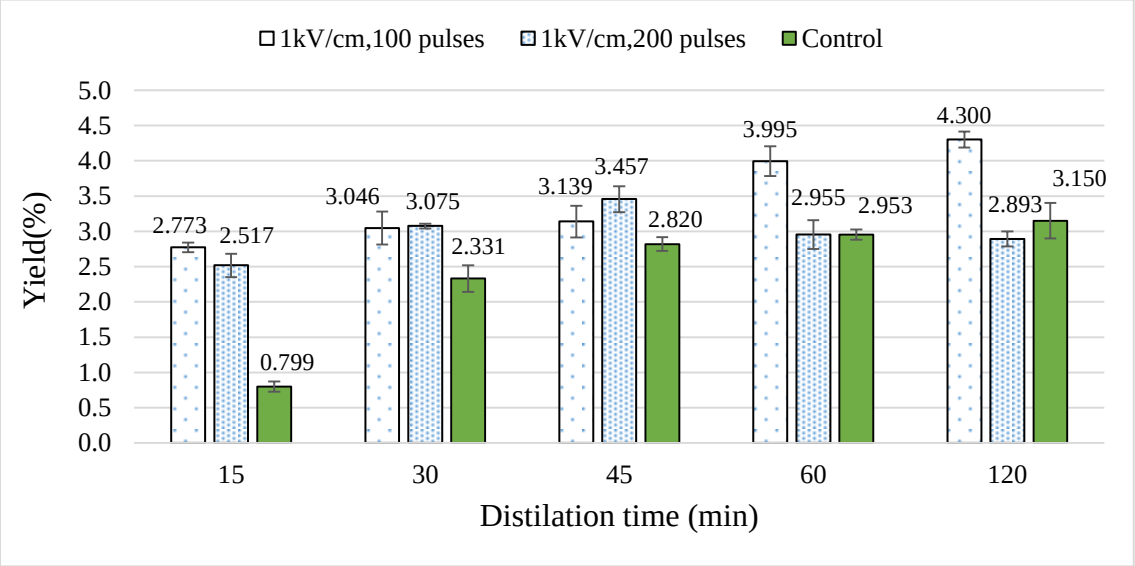

Fig.3. Yield of EO from *Lavandula angustifolia* with PEF treatment (1 kV/cm).

Following these results, the PEF treatment reduced the energy consumed by the distillation process by 50% since we obtained in 30 min of distillation with PEF treatment a quantity equal to or greater than the quantity of oil obtained in 60 min of distillation by the conventional method.

This energy reduction significantly influences the cost of producing and selling essential oil. These results are consistent with other work that have used PEF treatment to intensify essential oils from different plants (Barros *et al*., 2022; Miloudi *et al*., 2018; Tintchev *et al*., 2012; Yajun *et al*., 2017).

The impact of the PEF on the oil yield is explained by the phenomenon of electroporation which causes a rupture of the cell membranes, making it possible to facilitate the permeability of the intracellular and therefore the transfer of mass (Zimmermann *et al*., 1974; Vorobiev and Lebovka, 2011; Angersbach *et al*., 2000). EO extraction yield obtained with PEF treatment with intensity of 1 kV/cm, 200 pulses was lower than that obtained with a PEF of 1 kV/cm, 100 pulses. This phenomenon was observed when the distillation time reached or exceeded 60 min. We did not observe a significant increase after applying PEF of 2 kV/cm, 100 and 200 pulses compared to the reference (Fig. 4) for distillation times equal to or greater than 30 min.

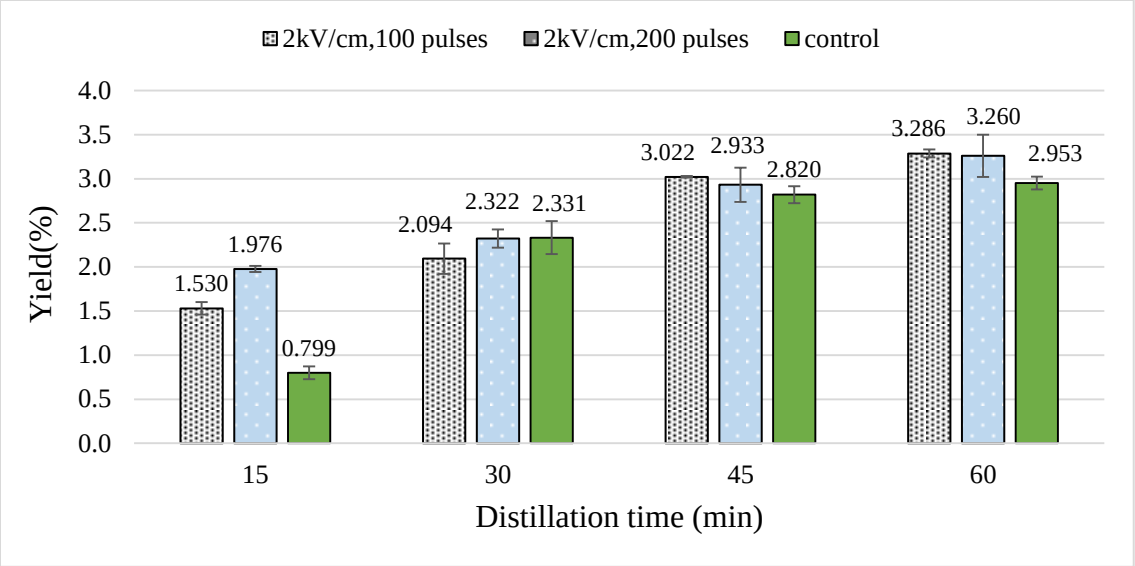

Fig.4. Yield of EO from *Lavandula angustifolia* with PEF treatment (2 kV/cm).

These results show that increasing the field intensity and the number of pulses (treatment time) has no impact on the oil yield because the high field intensity and the long treatment damage the cells, which explains the low yields observed with PEF of 2 kV/cm, 100 and 200 pulses which is in agreement with the behavior observed by other authors (Vorobiev and Lebovka, 2008). The highest increase in essential oil yield was achieved when PEF of 1 kV/cm and 100 pulses were applied. The yield increased by 3.046% for 30 min of distillation (conventional method = 2.331%) until 3.995% for 60 min of distillation (conventional method = 2.953%).

We observed a significant increase of 31.16% when the distillation time goes from 30 min to 60 min and a non-significant increase of 7.63% when the distillation time goes from 60 min to 120 min where we obtained a maximum yield of 4.3% (conventional method = 3.150%) which leads to choosing 1 hour of distillation as the optimal duration to obtain maximum yield of the EO from *Lavandula angustifolia* after treatment with PEF of 1 kV/cm, 100 pulses.

Effect of PEF treatment on pH of extracted essential oil

A pH study was made to observe the influence of PEF on the degradation of EO from *Lavandula angustifolia*. The results of this study are shown in Fig. 5. For this study, we used the same protocol and operating conditions.

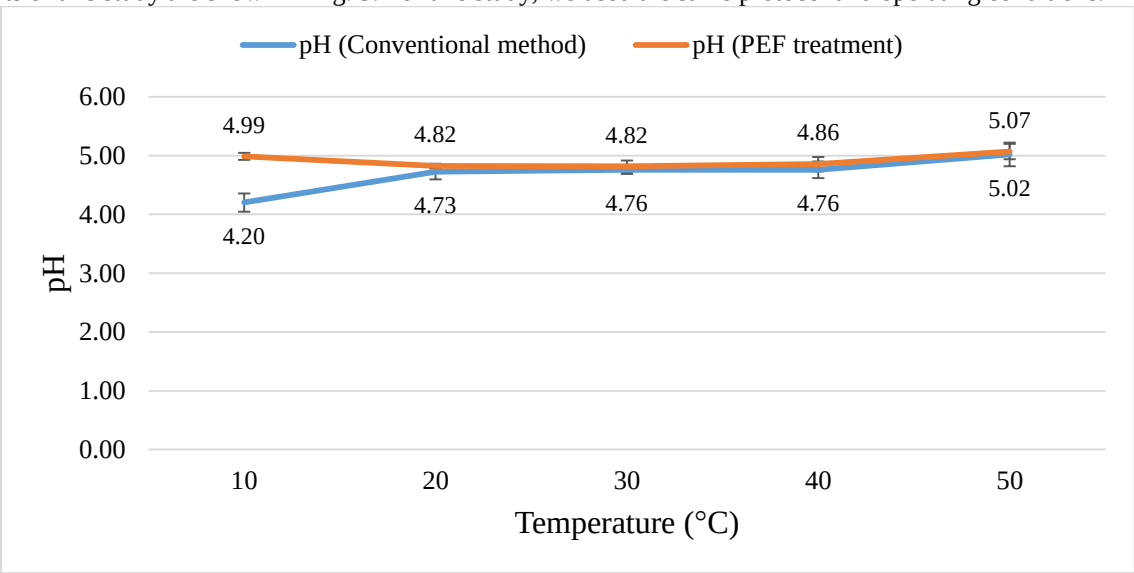

Fig.5. Comparison between pH of EO from *Lavandula angustifolia* extracted by the conventional method and after PEF treatment.

To follow the pH for a control sample and a sample treated with PEF, temperatures of 10°, 20°, 30°, 40° and 50° were applied. The results show that PEF did not affect the chemical properties of the essential oil. Therefore, the PEF treatment is a safe method for obtaining the essential oil since it did not cause any change in pH.

The interesting observation is that the pH of the PEF-treated sample is more stable than the control sample from the point where the temperature was 10° to the temperature of 50°. We also observed that the pH values of the PEF-treated sample were slightly higher than the values of the control sample.

Effect of PEF on anti-inflammatory activity

For the anti-inflammatory activity of EO from Lavandula angustifolia, HRBC (Human Red Blood Cell membrane) stabilization results showed non-dose-dependent HRBC membrane stabilization activity for both samples (conventional method and PEF). In addition, the stabilizing activity exerted by the PEF sample of *Lavandula angustifolia* EO ((Fig.6) was superior to that of conventional EO and diclofenac sodium.

The results of the HRBC stabilization activity of the EO treated with PEF were 24.11 ± 0.57% at 500 µg/mL and 24.43 ± 0.07% at 1000 µg/mL. In addition, authors have shown the antioxidant and anti-inflammatory characteristics of EO from *Lavandula angustifolia* (Pokajewicz *et al*., 2022).

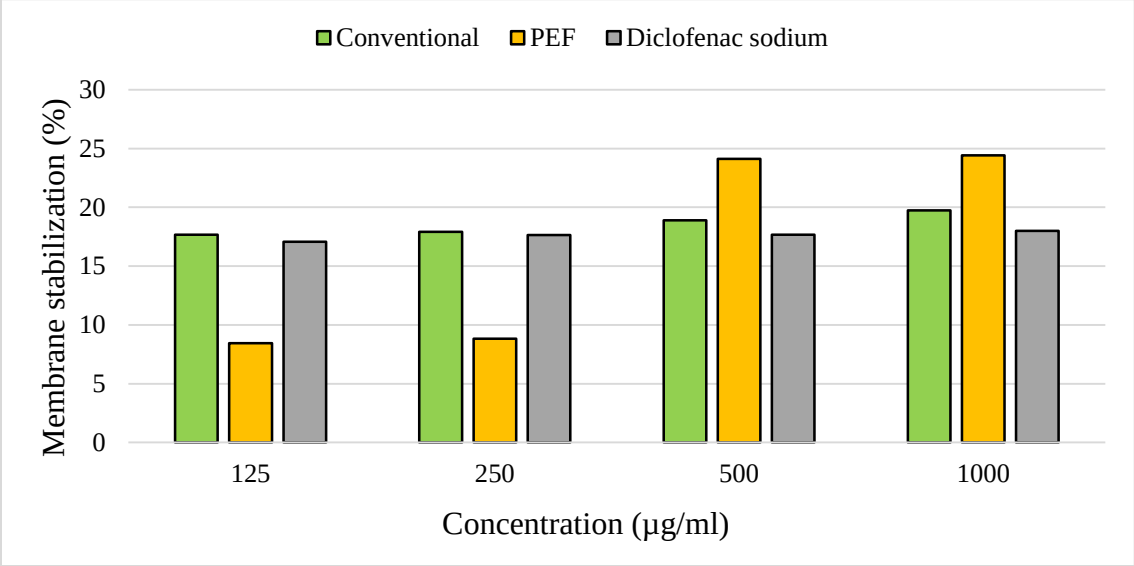

Fig. 6. Effect of PEF on anti-inflammatory activity.

Effect of PEF on the composition of essential oil from *L. Angustifolia*

Table 1 presents the principal components (>0.3%) of the essential oil of *Lavandula angustifolia* extracted by the conventional method (60 min of distillation) and the PEF treatment method (1kV/cm, 100 pulses, 60 min). The GC/MS analysis (Table 1) revealed quantitative but not qualitative differences between the two EOs according to conventional and PEF.

The main components are linalool: 13.05% - 10.50% (PEF), camphor: 8.86% - 6.38% (PEF), linalool oxide: 7.54% - 6.16% (PEF), linalool acetate: 5.93% - 7.60% (PEF) ,borneol: 5.56% - 4.53% (PEF), eucalyptol: 4.54% - 3.86% (PEF) and hexyl butyrate: 0.93% - 1.00% (PEF). Similar components of EO from *Lavandula angustifolia* have been reported by other authors (Ciobanu *et al*., 2012) : linalool (9.68%), linalool oxide (4.1%), linalool acetate (3.23%), camphor (8.35%), eucalyptol (2.46%).

Previous studies indicated that linalool and Linalool acetate, fenchone, eucalyptol, and borneol are major compounds in the EO from *Lavandula angustifolia* (Andrei *et al*., 2018; Bogdan *et al*., 2020; Tardugno *et al*., 2019). The chemical composition influences the biological properties of EOs (Andrei *et al*., 2018). Relatively low temperatures facilitate the formation of Linalool. The concentration of Linalol acetate determines the quality of the lavender essential oil used in perfumery, with larger quantities associated with better quality of perfume; On the other hand, the concentration of Linalool influences the antimicrobial properties of the EO (Bogdan *et al*., 2020).

Table 1. Major Chemical Compounds.

Chemical Compounds*	Molecular Formula	Control	PEF
Linalool	$C_{10}H_{18}O$	13.05	10.50
Camphor	$C_{10}H_{16}O$	8.86	6.38
Linalool, oxide	$C_{10}H_{18}O_2$	7.54	6.16
Linalool acetate	$C_{12}H_{20}O_2$	5.93	7.60
Borneol	$C_{10}H_{18}O$	5.56	4.53
Eucalyptol	$C_{10}H_{18}O$	4.54	3.86
Hexyl butyrate	$C_{10}H_{20}O_2$	0.93	1.00
Nerol	$C_{10}H_{18}O$	0.57	0.54
Carvone	$C_{10}H_{14}O$	0.57	-
Hexyl acetate	$C_8H_{16}O_2$	0.54	0.40
3-Octanone	$C_8H_{16}O$	0.53	0.33
Lavandulyl acetate	$C_{12}H_{20}O_2$	0.50	0.45
Nerol acetate	$C_{12}H_{20}O_2$	0.50	0.50
Limonene oxide	$C_{10}H_{16}O$	0.48	-
2,6-Dimethyl-3,5,7-octatriene-2-ol, ,E,E-	$C_{10}H_{16}O$	0.42	0.23
Lavandulol	$C_{10}H_{18}O$	0.41	0.49
Spatulenol	$C_{15}H_{24}O$	0.41	-
Linalyl oxide	$C_{10}H_{18}O_2$	0.38	-
Carvacrol	$C_{10}H_{14}O$	0.37	0.32
Cryptone	$C_9H_{14}O$	0.37	0.31
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	$C_{12}H_{20}O_2$	0.36	0.41
Amitinol	$C_{10}H_{16}O$	0.32	0.21
Bornylformate	$C_{11}H_{18}O_2$	0.31	0.11
Hotrienol	$C_{10}H_{16}O$	0.31	0.42
Propanamide, 3-[4-(2-hydroxyethyl)piperazin-1-yl]-N-(3-bromophenyl)-	$C_{15}H_{22}BrN_3O_2$	0.30	-
Amyl vinyl carbinol acetate	$C_{10}H_{18}O_2$	0.26	0.41
Lavender lactone	$C_7H_{10}O_2$	0.25	0.44
Anhydrolinalool oxide	$C_{10}H_{16}O$	0.23	0.38
n-Hexanol	$CH_3(CH_2)_5OH$	0.21	0.37
Limonene	$C_{10}H_{16}$	0.19	0.44
Myrcene	$C_{10}H_{16}$	0.18	0.34
Terpinolene	$C_{10}H_{16}$	0.13	0.36
p-Chlorothioanisole	C_7H_7ClS	-	0.40
3,7-Dichloro-2-[4-chlorophenyl]-4-quinolinecarboxylic acid	$C_{16}H_8Cl_3NO_2$	-	0.33
Hexyl isobutyrate	$C_{10}H_{20}O_2$	-	0.33
Piperidinooxy, 4-hydroxy-2,2,6,6-tetramethyl-, acetate (ester)	$C_{11}H_{20}NO_3^+$	-	0.33

*Area > 0.3%
(-) absence of a compound

Similarly, the absence of specific compounds has also been reported in other studies. The composition of the EO is different depending on genetic and environmental factors. Also, the culture medium applied affected the percentage composition of each essential oil constituent (Wesołowska *et al*., 2019).

However, most oil obtained from plants grown in North Africa contained 1,8-cineole and camphor as the main constituents. 1,8-cineole and borneol dominate in essential oils isolated from the leaves of *Lavandula angustifolia* harvested near Isfahan, Iran. Moreover, the quality of EO depends on both a high content of linalool, linalool acetate and their mutual proportions (preferably greater than 1) (Wesołowska *et al*., 2019). Italian *Lavandula angustifolia* EOs are rich in linalool (35–36%), linalool acetate (12–21%), camphor (5–11%), 1,8-cineole (3–10%), terpinen-4 - ol (2–6%), farnesene (1–4%) and borneol (2–4%) (Caprari *et al*., 2021). Chemical, physical, and biochemical changes due to the action of light and temperature can influence the oil quality (Roulier and Roulier, 2007). The changes in the composition of the essential oil could come from several environmental (climatic, seasonal, geographical) and genetic differences (Jianu *et al*., 2013; Verma *et al*., 2010).

PEF did not affect the quality of the oil. Other minor components (<1.0 and >0.3%) identified in the EO of *Lavandula angustifolia* have been widely studied due to their commercial interest in the perfume industry, aromatherapy and in pharmaceuticals for its therapeutic effects as antibacterial, antiviral and sedative agent (Da Porto *et al*., 2009; Kim and Lee, 2002).

Linalool, with sweet, intense floral notes woody smell reminiscent of the lily of the valley, is used in flavors and perfumes in the form of its esters. This acts as a sedative and antiseptic (Kiran Babu *et al*., 2016). linalool enhances the antimicrobial activity of several essential oils (Tardugno *et al*., 2019). it has proven to be the most potent active ingredient against many microorganisms (Caprari *et al*., 2021). Linalool (10.50% ,PEF) is an aromatic monoterpene, also identified in high amounts in other aromatic herbs(Caprari *et al*., 2021).

Linalool acetate is used in decorative cosmetics, fine perfumes, shampoos, toilet soaps and other non-toiletries products as well as non-cosmetic products, namely, household cleaners and scented ingredient detergents (Kiran Babu *et al*., 2016). Linalool acetate is an oxygenated monoterpene with an antimicrobial effect (Cavanagh and Wilkinson, 2002).

Borneol, having a "dry-camphorous, woody-pepper smell" along in its acetate and ketone (camphor) form, adds to the distinctive warm, minty, herbaceous aroma of typical lavender (Kiran Babu *et al*., 2016). Borneol, also a monoterpene, has anti-inflammatory, analgesic, sedative, anti-nociceptive, vasorelaxant and antithrombotic effects (Oroian *et al*., 2019). Borneol, a widely used food and cosmetic additive, has analgesic, anti-inflammatory, and antibacterial properties. It is well known that 1,8-cineole and camphor are responsible for the insecticidal activity of Genus *Lavandula* plants (Wesołowska *et al*., 2019).

With its specific camphor odor, Camphor is used commercially as a moth repellent and as a preservative in pharmaceuticals and cosmetics (Wesołowska *et al*., 2019). It has numerous pharmaceutical applications, being considered as antiseptic, anti-inflammatory, anti-infectious, topical analgesic, antipruritic, antispasmodic, light expectorant, rubefacient, antitussive, nasal decongestant, and even contraceptive (Oroian *et al*., 2019).Other authors believe that the greater amount of camphor is less appreciated in perfumery but more effective as an antimicrobial (Pokajewicz *et al*., 2022). Additionally, eucalyptol has been shown to exhibit antimicrobial properties (Jianu *et al*., 2013).

CONCLUSION

PEF treatment at 1 kV/cm, 100 pulses proved to be an effective treatment method for the extraction of EO from *Lavandula angustifolia*, with a reduction in energy consumption of approximately 50%. The extraction yield after PEF treatment was 3.045% in 30min of distillation, while almost the same amount (2.953%) was obtained in 60 min of distillation by the conventional method. This study shows that 60 min was the optimal distillation time to extract the maximum of EO from *Lavandula angustifolia* after PEF treatment. For the same distillation time (60 min), the yield obtained by the conventional method will be lower by 35% compared to that obtained after PEF treatment.The GC-MS analysis showed the good quality of EO obtained after PEF treatment because it contains the main compounds that can be decisive in the pharmacological properties and industrial application. The variable composition of EO of *Lavandula angustifolia*suggest that this plant may lead to various biological and antioxidant activities. The evaluation of the antioxidant activity of *Lavandula angustifolia* carried out by the two methods showed a high antioxidant activity. The results obtained in this study prove that PEF could be an appropriate technology to improve the yield of EO, save energy and could open new perspectives for their industrial application.

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CONFLICT OF INTEREST

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

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