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Optimization of Pulsed Electric-Field-Based Total Polyphenols' Extraction from *Elaeagnus pungens* 'Limelight' Leaves Using Hydroethanolic Mixtures

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Abstract: In this study, the use of pulsed electric field (PEF) for the extraction of polyphenols from mature *Elaeagnus pungens* 'Limelight' leaves is discussed. Optimization of the main parameters that affect the extraction process was carried out. More specifically, the composition of the solvent (ethanol, water, and mixtures of the two at a 25% v/v step gradient) and the main PEF-related parameters (i.e., pulse duration, pulse period, and electric field intensity) was optimized. The obtained extracts were examined for their polyphenol content with the Folin–Ciocalteu assay and individual polyphenols were also assessed with high-performance liquid chromatography. The extracts obtained with PEF were compared to the extract compared without PEF, in terms of total polyphenols. According to the results, the optimum extraction parameters were found to be a pulse duration of 10 μs, a pulse period of 1000 μs, and an electric field intensity of 0.85 kV cm^{-1} after 20 min of extraction. The optimum solvent was found to be the 50% (v/v) ethanol/water mixture. The extract prepared under the optimum conditions was found to contain 58% more polyphenols compared with the extract prepared without PEF. Moreover, an increase of up to 92% was recorded for specific polyphenols. Based on the above, it was evidenced that the examined parameters influenced the recovery of polyphenols, suggesting that such parameters should be also examined in similar studies, in order to maximize the extraction yield of polyphenols.

Keywords: pulsed electric field; extraction; polyphenols; *Elaeagnus pungens*; Folin–Ciocalteu; HPLC-DAD-MS

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1. Introduction

As plants are constantly exposed to ultraviolet light, they produce metabolites in order to protect themselves from the harmful effects. One of the main classes of compounds widely found in plants is polyphenols [1]. Owing to the high polyphenolic content of their leaves and berries, their antioxidant and antimicrobial activities, the good resilience of the plant, and their no-known enemies, *Elaeagnus* species are currently attracting the interest of the research community and functional, food-making enterprises [2–6].

Among the plants of the *Elaeagnus* species, *Elaeagnus pungens* 'Limelight' is under-researched. It is a species of flowering plant in the *Elaeagnaceae* family often referred to by the names "oleaster" and "thorny olive". It is a species that has been brought to the southeastern United States and Europe from Asia, where it is native, including China and Japan. It is a typical ornamental and landscaping plant, which, occasionally, can also be considered as an invasive species. *E. pungens* is a densely branched shrub with a height and width of over 7 m and 4 m, respectively. Despite its potential for invasion, temperate regions cultivate *E. pungens* extensively as a garden plant as it is well adapted to the environmental conditions [2–6].

Extraction of polyphenols from plants is an ongoing trend, with more and more studies being published [2–4]. Recently, our group demonstrated the effectiveness of pulsed

electric field (PEF) as a standalone extraction method for polyphenols from plants [7]. This extraction method has several advantages, including, but not limited to (I) minimal environmental impact owing to its low energy consumption; (II) minimum damage to heat-sensitive substances because no heat is generated during PEF extraction; (III) different composition of the extracts, depending on the experimental parameters (e.g., electric field strength, pulse duration, pulse period, pulse frequency, and so on); and (IV) increased extraction yields in comparison with other techniques [8–10]. The unique aspect of PEF is the electroporation that occurs, rendering the cell membrane of the plants more permeable and, as such, making the diffusion of compounds easier. The preparation of extracts with PEF, employed in the food and pharmaceutical industries, is gaining popularity owing to all of the above-listed advantages [8]. To date, PEF has been used for the extraction of chemicals from plant material in numerous studies, including the extraction of polyphenols from Merlot grapes [11], olive leaves [12], tea leaves [13], citrus fruits [14], and potato peels [15].

In this study, we aimed to examine the extraction of polyphenols from *E. pungens* using PEF. This *Elaeagnus* species was chosen because it is under-researched. To maximize the extraction yield, the main parameters that can be tuned on PEF were optimized. Moreover, as the kind of solvent employed can impact the effectiveness of PEF extraction [8], and not much research has focused on this (water is typically the only solvent utilized in research [7,16–19], although a combination of water and ethanol results in greater extraction yields of polyphenols [20]), we also examined the use of water, ethanol, and their mixtures, so as to further maximize the extraction yield of polyphenols. Overall, we aimed to develop and propose a technology that would replace conventional extraction by drastically reducing the use of organic solvents and the energy input toward a more effective, efficient, and environmentally friendly polyphenolic compound isolation technique in an economical manner.

2. Materials and Methods

2.1. Chemicals

Water, ethanol, and acetonitrile were of HPLC grade. Ethanol (99.8%) and the Folin–Ciocalteu reagent were obtained from Panreac (Barcelona, Spain). Acetonitrile and formic acid were purchased from Carlo Erba (Val de Reuil, France), while anhydrous sodium carbonate (>99%) and gallic acid monohydrate were from Penta (Prague, Czech Republic). The HPLC reference substances luteolin-7-*O*-glucoside and *p*-coumaric acid were bought from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant Material Handling

The mature leaves of *Elaeagnus pungens* 'Limelight' were collected in mid-July 2021 from a 12-year-old shrub in the Karditsa Region of Greece (at 39°21′56″ N and 21°55′43″ E, with an elevation of 105 m, according to Google Earth version 9.124.0.1, Google, Inc., Mountain View, CA, USA).

The *Elaeagnus* branches were gathered early in the morning and transported to the laboratory 10 min later for quick processing. Following the removal of the branch, the leaves were thoroughly washed with tap water and dried with filter paper at room temperature until no moisture remained on their surface. Before each extraction, the leaves were ground for two minutes in a blender (Camry, Warsaw, Poland) using the same shear input and batch quantities to establish uniformity of the pulverization output and a minimal temperature increase. According to sieve analysis, the latter produced fibrous powders with an approximate average particle diameter of 0.8 mm (d10 = 360 μm, d50 = 750 μm, and d90 = 1120 μm).

2.3. Instrumentation

2.3.1. PEF System and Calculus

The PEF system utilized is the same as that previously demonstrated by Pappas et al. [21]. It is a static bench-scale system made of a pair of specially made stainless steel rectangular

treatment chambers, a high voltage (0.1–25 kV) power generator, a 25 MHz function/arbitrary waveform generator, and a customized electronic switch circuit (series of insulated gate bipolar transistors—IGBTs) (Val-Electronic, Athens, Greece). The rectangular extraction cells have capacities of 80 mL and are made up of two identical flat parallel stainless-steel plates measuring 10 cm by 10 cm, separated by a single piece of "Π"-shaped Teflon that serves as an insulator.

In our previous work [21], full details are given for the set of equations used to calculate the electric field intensity (E), the overall PEF treatment time ($t_{\text{PEFtreatment}}$), and the specific energy input W_{spec} (kJ kg^{-1}). A predetermined number of pulses (N) specified by the extraction duration ($t_{\text{extraction}}$) and the period were produced by the pulse generator as unipolar, rectangular pulses with pulse durations (t_{pulse}) ranging from 1, 10, and 100 μs under a period (T) of 100 and 1000 μs.

2.3.2. Absorbance Measurements

The absorbance measurements were performed using a Shimadzu spectrophotometer (UV-1700, Shimadzu Europa GmbH, Duisburg, Germany).

2.3.3. HPLC System

A Finnigan AQA mass spectrometer (San Jose, CA, USA) coupled to a P4000 pump and a UV6000LP diode array detector were used for the chromatographic separation and detection of the compounds. A Phenomenex Luna C18 column (5 μm, 4.6 mm × 250 mm) from Phenomenex, Inc. (Torrance, CA, USA) was used as the stationary phase. It was kept in an oven to maintain a constant temperature of 40 °C throughout all runs. The mobile phase was composed of (A) water with formic acid (0.5% v/v) and (B) acetonitrile and water (60:40) with formic acid (0.5% v/v). The gradient elution method employed was as follows: 5% B to 40% B in 40 min, followed by 50% in 10 min, then 70% in 10 min, and kept constant for a further ten minutes. The flow rate was 1 mL min^{-1} and the duration was 70 min in total. A rheodyne injector was used for sample injections (the sample volume was 20 μL).

2.4. Dry Weight Determination

Each batch of ground leaves was weighed appropriately before and after drying to a constant weight at 105 °C using an oven to determine the water content of the leaves (Binder BD56, Bohemia, NY, USA). The proportion of moisture and volatiles content was then determined using Equation (1) as follows:

$$\%\ \text{Moisture and volatiles content} = \frac{W_{\text{BD}} - W_{\text{AD}}}{W_{\text{BD}}} \times 100 \quad (1)$$

where W_{BD} represents the weight (g) of leaf powder before drying and W_{AD} represents the weight (g) of leaf powder after drying. The leaves contained about 50% (w/w) moisture and volatiles. Each sample's dry matter (g) was calculated using Equation (2):

$$\text{Dry matter} = W_{\text{S}}\ \frac{(100 - \%\ \text{Moisture and volatiles content})}{100} \quad (2)$$

where W_{S} is the weight (g) of freshly cut leaf powder.

2.5. Experimental Design and Extraction Processing Steps

To increase the polyphenolic content of the extracts, the major PEF variables were thoroughly screened to identify the optimal PEF ones for the particular system (plant material and solvent). The primary PEF parameters that control permeability were determined to be field intensity (E), pulse duration (t_{pulse}), and pulse period (T) for a particular extraction duration ($t_{\text{extraction}}$). The uniform electric field chambers expose every cell in the sample to the same electric field, which is useful for electroporation. If the field strength is adequate and close to the optimal value, high-yield intracellular extraction of compounds is possible. The electric field strength set point must be chosen via organized experimental design

because the best extraction yields might vary significantly above or below the optimal value. Based on the literature [22], we decided to maintain the specific energy input level below 5 kJ kg^{-1} and evaluate the electric field between 0.7 and 1 kV cm^{-1} for the best extraction of bioactive compounds from *Elaeagnus* leaves.

The study's design was divided into groups and parameters for process optimization were tuned one at a time so that the results of each group of experiments provided input for the ones that followed. Table 1 displays the experiments that were performed. Experiments were divided into three groups: the field intensity effect determination (group 1), the pulse and period duration influence (group 2), and the impact of the type of hydroethanolic combination chosen as the extraction solvent (group 3).

Table 1. Design of the PEF process optimization research; "-" denotes no values for control samples (no PEF applied).

Exp. Group	Exp. Series	EtOH:H_2O Content	$t_{extraction}$ (min)	E (kV cm^{-1})	t_{pulse} (μs)	T (μs)	N	$t_{PEFtreatment}$ (s)	Energy Input (kWh)	Specific Energy Input (kJ kg^{-1})
1	1	0%	20	1	10	1000	1.20×10^6	12	1.68×10^{-6}	8.85×10^{-2}
	2	0%	20	0.85	10	1000	1.20×10^6	12	1.43×10^{-6}	7.52×10^{-2}
	3	0%	20	0.7	10	1000	1.20×10^6	12	1.18×10^{-6}	6.20×10^{-2}
	4	0%	20	-	-	-	-	-	-	-
2	5	0%	20	0.85	1	100	1.20×10^7	12	1.43×10^{-6}	7.52×10^{-2}
	6	0%	20	0.85	10	1000	1.20×10^6	12	1.43×10^{-6}	7.52×10^{-2}
	7	0%	20	0.85	100	1000	1.20×10^6	120	1.43×10^{-5}	7.52×10^{-1}
	8	0%	20	-	-	-	-	-	-	-
3	9	0%	20	0.85	10	1000	1.20×10^6	12	1.43×10^{-6}	7.52×10^{-2}
	10	0%	20	-	-	-	-	-	-	-
	11	25%	20	0.85	10	1000	1.20×10^6	12	1.43×10^{-6}	7.52×10^{-2}
	12	25%	20	-	-	-	-	-	-	-
	13	50%	20	0.85	10	1000	1.20×10^6	12	1.43×10^{-6}	7.52×10^{-2}
	14	50%	20	-	-	-	-	-	-	-
	15	75%	20	0.85	10	1000	1.20×10^6	12	1.43×10^{-6}	7.52×10^{-2}
	16	75%	20	-	-	-	-	-	-	-
	17	100%	20	0.85	10	1000	1.20×10^6	12	1.43×10^{-6}	7.52×10^{-2}
	18	100%	20	-	-	-	-	-	-	-

Ten grams of plant leaves powder was mixed with 60 mL of the extraction solvent to extract the total polyphenols (at a ratio of 6:1 mL g^{-1}) into the PEF treatment chamber. All experiments were conducted at an ambient temperature of 22 ± 1 °C. The temperature of the treatment chamber's contents was measured before and after each extraction run using an infrared thermometer (GM300, Benetech, Shenzhen Jumaoyuan Science and Technology Co., Ltd., Shenzhen, China). In every PEF-assisted extraction run, the temperature increments due to the treatment were always under 1 °C. After each extraction, the mixtures were removed from the PEF chamber and placed in a Falcon tube and centrifuged (9164× g for 10 min at room temperature) to separate the plant material. The supernatants were retracted and immediately analyzed, as described in Sections 2.6 and 2.7. Comparisons between them and control extracts (obtained without the application of PEF) were used to assess the effect of each examined parameter. All extractions were carried out three times.

2.6. Folin–Ciocalteu Assay

The total polyphenol content (TPC) of the extracts was evaluated using a previously described technique [21,23]. In brief, the plant extracts were diluted at a ratio of 1:50 with a formic acid solution (0.5% v/v) (plant extract/formic acid solution). Following that, 100 μL of the diluted sample and 100 μL of the Folin–Ciocalteu reagent were combined and vortexed. The addition of 800 mL of a sodium carbonate solution (5% w/v) followed after 2 min. The absorbance of the solution was measured at 740 nm after incubation for 20 min at 40 °C. A suitable calibration curve was created using gallic acid and the results were expressed as mg of gallic acid equivalents (GAEs) per g of dry weight.

2.7. HPLC-Based Determination of Polyphenols

Samples were injected into the HPLC-DAD-MS (ESI+) system. Tentative identification of the compounds was based on their mass spectra and previous reports. Quantification was carried out using calibration curves using luteolin-7-*O*-glucoside and *p*-coumaric acid. The results were expressed as the concentration (mg g^{-1}) for each group of identified components.

2.8. Statistical Analysis

All extracts were prepared in triplicates and three replicate analyses were performed, resulting in a total of nine measurements. The results are presented as the average of all measurements ± standard deviation (SD). After ensuring that the data were not normally distributed using the Shapiro–Wilk test, statistically significant differences were assessed using the Kruskal–Wallis test for $p < 0.05$ using SPSS (SPSS Inc., Chicago, IL, USA) software.

3. Results and Discussion

A novel aspect of this study is the emphasis on PEF optimization as a standalone solid–liquid extraction method for bioactive components. In order to increase the polyphenols' extraction yield from *Elaeagnus* leaves, several PEF settings and extraction solvents were examined. The average humidity of the leaves was found to be 60 ± 2%. The primary PEF parameters were thoroughly pre-screened in order to determine the optimal PEF parameter range for the particular system (plant material and solvent) and to increase the polyphenol content of the extracts. We pre-examined the permeability regulating factors, including field intensity (E), pulse length (t_{pulse}), and pulse period (T), for various extraction times as the primary PEF parameters ($t_{extraction}$).

Our starting point (fixed variables) from the preliminary study, which is not presented here, was the range of the electric field strength (E) between 0.7 and 1 kV cm^{-1}, pulse duration (t_{pulse}) between 1 and 100 µs, pulse period (T) between 100 and 1000 µs, and a fixed extraction time ($t_{extraction}$) of 20 min. There are significant discrepancies between the various PEF settings and extraction solvents, as shown in the result overview in Table 2.

Table 2. Mean values of *Elaeagnus* leaves extracts' TPC (mg GAE g^{-1} dw); each Exp. section means inside rows with a distinct superscript letter (a–g; A–C) differ significantly ($p < 0.05$). "-" denotes no values for control samples (no PEF applied).

Exp. Group	Exp. Series	Average TPC (mg GAE g^{-1} dw)	% Increase
1	1	12.76 ± 0.03^{b}	28.7 ± 0.3^{B}
	2	14.38 ± 0.05^{a}	45.0 ± 0.5^{A}
	3	12.96 ± 0.23^{b}	30.7 ± 2.4^{B}
	4	9.92 ± 0.07^{c}	-
2	5	13.16 ± 0.21^{c}	32.7 ± 2.1^{C}
	6	14.38 ± 0.05^{a}	45.0 ± 0.5^{A}
	7	13.53 ± 0.09^{b}	36.4 ± 0.9^{B}
	8	9.92 ± 0.07^{d}	-
3	9	14.38 ± 0.05^{d}	45.0 ± 0.5^{B}
	10	9.92 ± 0.07^{f}	-
	11	19.7 ± 0.88^{b}	$54.7 \pm 6.9^{A,B}$
	12	12.74 ± 0.91^{e}	-
	13	23.32 ± 1.72^{a}	57.5 ± 11.6^{A}
	14	14.8 ± 0.74^{d}	-
	15	17.43 ± 0.17^{c}	$47.6 \pm 1.4^{A,B}$
	16	11.81 ± 0.09^{e}	-
	17	8.13 ± 0.36^{g}	15.1 ± 5.2^{C}
	18	7.07 ± 0.02^{g}	-

3.1. Effect of PEF Electric Field Intensity (Group 1)

Three different input values for the electric field strength were investigated for this optimization section: 1, 0.85, and 0.7 kV cm^{-1} (group 1). According to Table 2, when three different levels of PEF electric field intensities were tested, the level of 0.85 kV cm^{-1} significantly improved the efficiency of the aqueous extraction of total polyphenols from *Elaeagnus* leaves using plain water at pulse durations of 10 μs and pulse periods of 1000 μs for a 20 min extraction period. The resulting extract's TPC was determined to be 14.38 ± 0.02 mg GAE g^{-1} dw at 0.85 kV cm^{-1}, higher than the two other settings by nearly 18% and the reference non-PEF treated sample by nearly 4%. The results are in accordance with our previous study, in which a similar increase in the TPC of the extract from fresh olive leaves was observed when the intensity was increased to 0.85 kV cm^{-1}, whereas a further increase in the intensity did not increase the TPC [21].

3.2. Effect of PEF Pulse and Period Duration (Group 2)

Given the optimal electric field strength, this group of experiments was carried out to investigate the most effective pulse-to-period PEF settings. The most effective PEF-assisted extraction settings depend on the specific energy delivered to the sample or the cell membrane relaxation time, which is altered by different pulse durations or periods. To verify the improvement in extraction efficiency and any selectivity of the components extracted, additional analysis of the polyphenolic profile using HPLC-DAD-MS was carried out for the control and the samples were produced under optimal conditions. Tables 2 and 3 show the outcomes, respectively.

Table 3. *Elaeagnus* leaves were extracted for 20 min with 0% EtOH, yielding a concentration of the major compounds (mg g^{-1} dw).

Exp. Series	Concentration Parameters	*p*-Coumarate Derivative [1]	1-Luteolin Rutinoside Derivative [2]	Luteolin Glucoside Derivative [2]	2-Luteolin Rutinoside Derivative [2]
5	Average [3] % Increase [3]	0.756 ± 0.04^{b} 27.2 ± 0.7^{B}	0.399 ± 0.015^{a} 49.2 ± 5.7^{A}	0.021 ± 0.001^{a} 25.6 ± 3.8^{A}	0.063 ± 0.001^{a} 41.8 ± 3.0^{A}
6	Average % Increase	1.075 ± 0.051^{a} 80.9 ± 8.5^{A}	0.402 ± 0.015^{a} 50.5 ± 5.6^{A}	0.023 ± 0.001^{a} 35.6 ± 6.8^{A}	0.060 ± 0.004^{a} 35.1 ± 8.1^{A}
7	Average % Increase	0.772 ± 0.042^{b} 30.0 ± 7.2^{B}	0.386 ± 0.013^{a} 44.5 ± 4.8^{A}	0.019 ± 0.001^{b} 9.6 ± 4.7^{B}	0.062 ± 0.002^{a} 41.4 ± 5.7^{A}
8	Average % Increase	0.594 ± 0.029^{c} - [4]	0.267 ± 0.017^{b} -	0.017 ± 0.001^{b} -	0.044 ± 0.001^{b} -

[1] Quantified as *p*-coumaric acid. [2] Quantified as luteolin-7-*O*-glucoside. [3] Means within each column (compound) with different superscript letters (a–c; A,B) are significantly ($p < 0.05$) different. [4] "-" denotes no values for control samples (no PEF applied).

From Tables 2 and 3, it can be seen that the results are similar. The clear overall choice was the settings of 10 μs pulse duration and 1000 μs pulse period. In particular, the TPC results presented in Table 2 exhibit an almost 10% yield increase when compared with the other two sets of PEF settings tested and a 45% yield increase when compared with the reference sample. Another observation is that a 10-fold power increase in the system (case of pulse duration 1 μs and pulse period of 100 μs) does not seem to have any effect on the extraction yield. This is valid for this static solid–liquid extraction study as it is in line with previous studies.

From the HPLC results, it was obvious that the choice of the setting (10 μs or 1000 μs) compared with the others results in an almost 270% yield increase in the Group 1 components, which represent almost 70% of the components identified. For the rest of the component groups, a low but monotonous significantly positive effect of PEF treatment is evident. As shown by the comparative difference in the compound concentration percentage increment on each PEF condition, it is clear that PEF conditions (such as t_{pulse} and T) have a nonlinear effect on the extraction rate of intracellular components, proving the

selectivity of this extraction method. The second assertion is supported by the findings and observations of our earlier study [21], in which we found that pulse impacted the rate at which the identified components were extracted, enabling the selective extraction of different molecules. A combination of molecular size and solubility effects must be considered for their selective extraction. This finding is significant as selective extraction is a difficult, laborious, and energy-intensive procedure.

3.3. Effect of the Hydroethanolic Mixture Choice as an Extraction Solvent (Group 3)

This section was designed to screen out the ideal ethanol/water ratio between the samples treated with PEF and the control. Tables 2 and 4 show the outcomes, respectively.

Table 4. Specific polyphenolic component groups' concentrations (mg g^{-1} dw) of the *E. pungens* extracts, obtained by applying five different hydroethanolic solvents during PEF extraction.

Exp. Series	Concentration Parameters	*p*-Coumarate Derivative [1]	1-Luteolin Rutinoside Derivative [2]	Luteolin Glucoside Derivative [2]	2-Luteolin Rutinoside Derivative [2]
9	Average [3] % Increase [3]	1.075 ± 0.003^{d} 80.9 ± 0.5^{A}	0.402 ± 0.029^{d} 50.5 ± 10.8^{B}	0.023 ± 0.002^{e} 35.6 ± 8.8^{A}	0.060 ± 0.002^{e} 35.1 ± 4.6^{B}
10	Average % Increase	0.594 ± 0.040^{f} - [4]	0.267 ± 0.006^{f} -	0.017 ± 0.001^{f} -	0.044 ± 0.003^{f} -
11	Average % Increase	1.321 ± 0.078^{b} 31.4 ± 7.8^{D}	$0.452 \pm 0.009^{b,c}$ 33.8 ± 2.8^{D}	0.036 ± 0.001^{c} 36.1 ± 4.6^{C}	0.072 ± 0.005^{c} 33.3 ± 8.5^{C}
12	Average % Increase	1.005 ± 0.037^{d} -	0.338 ± 0.022^{e} -	$0.026 \pm 0.002^{d,e}$ -	0.054 ± 0.002^{e} -
13	Average % Increase	1.604 ± 0.087^{a} $37.3 \pm 7.4^{B,C}$	0.646 ± 0.025^{a} 91.9 ± 7.3^{A}	0.056 ± 0.004^{a} 37.9 ± 9.7^{A}	0.088 ± 0.002^{a} 51.7 ± 3.3^{A}
14	Average % Increase	1.168 ± 0.029^{c} -	0.336 ± 0.017^{e} -	0.041 ± 0.002^{b} -	0.058 ± 0.004^{e} -
15	Average % Increase	0.870 ± 0.048^{e} 31.8 ± 7.2^{C}	0.478 ± 0.011^{b} 12.4 ± 2.6^{C}	0.053 ± 0.003^{a} $27.3 \pm 7.3^{A,B}$	0.083 ± 0.005^{b} 26.9 ± 7.7^{B}
16	Average % Increase	0.660 ± 0.048^{f} -	$0.426 \pm 0.015^{c,d}$ -	0.042 ± 0.001^{b} -	0.065 ± 0.003^{d} -
17	Average % Increase	0.479 ± 0.016^{g} 43.0 ± 4.9^{B}	0.237 ± 0.005^{g} 53.9 ± 3.5^{B}	0.034 ± 0.002^{c} 16.1 ± 5.8^{B}	0.045 ± 0.002^{f} 47.1 ± 7.2^{A}
18	Average % Increase	0.335 ± 0.022^{h} -	0.154 ± 0.006^{h} -	0.029 ± 0.002^{d} -	0.031 ± 0.001^{g} -

[1] Quantified as *p*-coumaric acid. [2] Quantified as luteolin-7-*O*-glucoside. [3] Means within each column (compound) with different superscript letters (a–h; A–D) are significantly ($p < 0.05$) different. [4] "-" denotes no values for control samples (no PEF applied).

From Tables 2 and 4, it can be seen that the results are quite similar. The clear overall choice was the 1:1 water to EtOH mixture. In particular, the TPC results presented in Table 2 conclude that the PEF effect appears to be at a maximum in the range of 25% to 75% EtOH/H_2O v/v ratio, with a peak at a 50% EtOH:H_2O v/v ratio. A 58% yield increase is achieved when utilizing the 1:1 v/v hydroethanolic mixture when compared with the PEF treated sample using plain water and at least 18% higher than any other hydroethanolic mixture of the PEF treated samples. A negative effect appears when pure EtOH is used as a solvent, both in the reference and in the PEF-treated samples. From the HPLC results presented in Table 4, it is observed that the most positively affected group of components is Group 3 (which represents 27% of the components identified), resulting in an increase of 92%. For the rest of the component groups, a low but monotonous significantly positive effect of PEF treatment is evident, even in the case of the pure ethanol solvent choice.

Numerous variables, including the solvent's polarity, the solubility of the compounds in the solvent, and the electrical conductivity, impact the extraction solvent choice in PEF [8]. Pure ethanol, used in this study, has an electrical conductivity of 0.1 (μS cm^{-1}), whereas water has a conductivity of 2.3 (μS cm^{-1}) [12]. As a result, the electrical conductivity

of the water/ethanol mixture drops as the volume of ethanol increases. As previously mentioned, the cell membrane electroporation during PEF extraction is positively altered by the solvent's higher electrical conductivity [8], leading to better compound extraction from the plant cells. This runs counter to the observation that the extracts' content in polyphenol compounds was higher when ethanol and water combinations were utilized, suggesting a different explanation for the observed outcomes.

The solvent's polarity could be a potential explanation. It is well known that ethanol has a polarity index of 5.2 and water has a polarity index of 10 [24]. As a result, the polarity diminishes as the proportion of ethanol in the mixture rises. The polarity index of the mixes can be calculated using a proper equation [25] (i.e., $P_m = R_1P_1 + R_2P_2$, where R_1 and R_2 are the volume fractions of solvents 1 and 2, respectively, and P_1 and P_2 are the polarity indices of the two solvents). A solvent with a higher polarity index can be used to extract more polar phenolic compounds, whereas a solvent with a lower polarity index, such as a mixture of 50% ethanol and water, can be used to extract polyphenols with a wider range of polarity. Because the plant's ability to swell decreases as the fraction of ethanol increases, the extraction efficiency falls, which can be used to explain why the TPC levels in the extracts obtained with 0% and 100% ethanol were identical. Contrarily, using plain water increases the solvent's polarity and reduces the extraction of less polar substances [26]. Our findings support earlier research showing that extracts produced by mixing ethanol and water contain higher polyphenols [20,25]. Furthermore, our results show that PEF extractions require other solvents rather than just water.

In our previous studies, we have demonstrated that PEF can enhance the extraction of polyphenols in many cases. For instance, the TPC of *Sideritis raiseri* was increased by up to 146% when PEF was employed, compared with the simple extraction method [27]. Similarly, in the case of *Aesculus carnea* extracts, PEF increased the TPC by up to 33% [28]. However, this was not the case with extracts prepared from grape stems [29]. The sole use of PEF before extraction resulted in a non-statistically significant increase in the TPC (~4%). However, when PEF was followed by US treatment, an increase of up to 35% was recorded. Moreover, in another study, it was also reported that PEF did not increase the TPC of *Thymus serpyllum* extract [16]. As such, it can be inferred that PEF has a major potential for increasing the extraction of bioactive compounds from various plants. However, its efficiency is not always granted and studies on each specific plant should be carried out to determine its efficiency.

4. Conclusions

According to our research, the use of PEF as an extraction technique, aided by "green" solvents, is efficient to extract specific bioactive compounds from fresh *Elaeagnus pungens* 'Limelight' leaves. Although the standalone extraction optimization technology for continuous flow industrial applications has inherent limitations, PEF offers an opportunity for environmentally friendly selective extraction of polyphenolic compounds from *Elaeagnus* leaves. Depending on the biomass characteristics, availability, and composition, PEF-based extraction technology can sustainably support the production of functional foods, producing high-quality products enriched in bioactive compounds that have a number of health benefits for the public. Our findings were clear that a 1:1 mixture of water and ethanol at 0.85 kV cm^{-1} and 10 µs pulse duration at a 1000 µs pulse period can boost the polyphenols mass in the extract by ~60% compared with a non-PEF-treated sample of the same extraction solvent. Complementary work is strongly advised in order to include the solvent, pH, and polarity effects in the PEF outcome in order to maximize the concentration of polyphenols. Future research should concentrate on further refining the PEF process parameters to maximize and validate the concentration of both selective and total polyphenols.

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