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Enhancement of Polyphenols Recovery from *Rosa canina*, *Calendula officinalis* and *Castanea sativa* Using Pulsed Electric Field

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Abstract: The current study evaluates the Pulsed Electric Field (PEF) technique for the extraction of polyphenols from the plants *Rosa canina*, *Calendula officinalis* and *Castanea sativa*. These plants are traditionally used both for the preparation of therapeutic decoctions and the aromatization of beverages (alcoholic or not). Pulses of 10 μs duration were used to apply electric field intensities ranging from 1.2 to 2.0 kV cm^{-1}. The period of the phenomenon was set to 1 ms, with a total extraction time of 20 min. The total polyphenol content as well as the identified polyphenolic compounds of the extracts were determined for monitoring and evaluation. To estimate the PEF effect, control extracts were prepared using the same process as PEF extracts but without the application of electric field. For all the three plant materials studied, the PEF technique appeared to be successful in increasing polyphenols extraction. The application of a moderate to high electric field, up to 1.4 kV cm^{-1}, resulted in increased total and individual polyphenols recovery, reaching 63.79% and 84%, respectively, in the case of *Rosa canina* fruits.

Keywords: Pulsed Electric Field; green extraction; phenolics; medicinal herbs

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1. Introduction

Medicinal plants comprise a variety of pharmaceutical properties due to the presence of various bioactive phytoconstituents that possess distinct mechanisms of action. Those bioactive phytoconstituents could further be utilized as potential alternatives to conventional drugs, a field that is gaining an important position and great demand globally as herbal formulations are safer, cheaper, and have negligible side effects, compared to their synthetic analogs [1,2].

The Swiss physician and chemist Paracelsus (1493–1541) expressed the basic principle of toxicology: Natural substances are the best healers, but all substances are poison, and it is only the correct dose which makes them a remedy [3]. Herbal products pose a number of risks (e.g., toxins, pesticides, heavy metals, alkaloids, saponins, etc.) [4–7], but other functional components having useful actions (i.e., antioxidant activity) show a great potential for illness prevention and treatment, as well as human health promotion [8]. The presence of plant toxins, their bioavailability from the matrix, and interactions with other inherent plant constituents, as well as the potential health benefits of foods (antioxidants and other natural protective agents), should be considered [5]. Nevertheless, consumers tend to favor the use of additives of natural origin attributing to artificial additives of various suspected undesirable actions of such as the promotion of carcinogenesis or mutagenicity [9].

Greece has a very large geographical area and a very rich diversity of medicinal herbs [10,11]. Among others, *Castanea sativa*, *Rosa canina* and *Calendula officinalis*, are well known for their therapeutic properties [12–14]. These three plants are used for the preparation of therapeutic decoctions (sweetened or unsweetened) or even for the aromatization of traditional beverages and foods. *R. canina* is also used for the preparation of traditional alcoholic beverages (liqueur).

C. sativa, a species of the Fagaceae family, can be found in southern Europe and Asia (China). Different properties such as antibacterial, DNA protection and prevention, and treatment of oxidative stress-mediated diseases have been attributed to *C. sativa* leaf extracts, while their infusion has been reported to treat cough, diarrhea, and rheumatic conditions [13]. The flavonoid rutin has been identified as the major component in *C. sativa* leaf extract. Other flavonoids, such as isoquercitrin and hyperoside, and phenolic acids, such as chlorogenic and ellagic, have been reported in significant amounts [15].

C. officinalis, known for its antibacterial, antiviral, anti-inflammatory, anti-tumor and antioxidant effects [12], is the only one among the 25 species of the genus Calendula that is broadly used clinically worldwide. *C. officinalis* healing and anti-inflammatory properties are listed in the World Health Organization. Derivatives of the flavonoids quercetin and isorhamnetin, all in the form of glycosides, are the main phenolic compounds in *C. officinalis* extracts. Phenylpropanoids such as 3-*O*-caffeoylquinic acid, caffeic acid and di-*O*-caffeoylquinic acids have also been identified in lower quantities [16].

R. canina has been studied for various antioxidant, anti-inflammatory, and diuretic properties. *R. canina* fruits (rose hips), the most used part of the plant, have been reported to treat respiratory infections such as cough, cold or sore throat, when infused, decocted, or consumed as syrup [14,17]. The major flavonoids present in rose hips have been found to be catechin and rutin. Other significant flavonoids observed in rose hips are eriocitrin, quercetin, apigenin, kaempferol, and naringenin. The phenolic acids caffeic, gallic, *p*-coumaric and ferulic have also been reported [18,19]. All these phenolic compounds have been shown to exhibit individually a wide range of therapeutic properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, anti-cancer, cardioprotective, and vasodilatory effects [20,21].

The development of a "green" and effective extraction method of phenolic compounds from their natural matrices is of great interest, as it could lead to the production of new drug precursors, as well as bio-functional foods and food supplements. Conventional extraction systems (or even newer extraction techniques, such as supercritical fluid extraction, ultrasonically assisted solvent extraction, accelerated pressurized and microwave-assisted extraction) involve the use of substantial mechanical force and/or thermal energy, the requirement of expensive equipment, costly and hazardous solvents for a long processing time, the evaporation of a huge amount of solvent, the low extraction selectivity, and the thermal decomposition of thermo labile compounds [22]. For example, the usual way to prepare herbal tea includes the plant material boiling in water or remaining in hot water for an extended period of time. This practice definitely reduces the extracted amounts of many important compounds [23]. The above drawbacks prevent systematic, industrial production of bioactive compounds using the above methods.

An emerging technique of which application has recently begun (on a large industrial scale) in the extraction of phytochemicals is the Pulsed Electric Field (PEF). Especially for liquid foods (such as oils, juices, and beverages incorporating juices), PEF treatment showed no substantial changes in the concentration of health-beneficial components (i.e., vitamins C and B, thiamine, riboflavin, retinol, cholecalciferol, a-tocopherol, etc.) as indicated by multiple studies reported by Nowosad et al. [24]. The principle of PEF is to disintegrate the cell membrane structure for increasing extraction. This is achieved by the application of an electric field that results to the formation of pores in weak areas of the cell membrane, causing a drastic increase of permeability. The external electric field increases the transmembrane potential and initiates the pore formation in the membrane of the biological cell. Once pores are created in the order of 0.5 nm radius, they may expand because of the

applied electric field [25]. The method to enhance permeability of cell membranes by applying external electrical force is known as electropermeabilization or electroporation [26]. Depending on the intensity of the electric field, the damage to the membrane is either reversible, or it is permanent and hence irreversible. Damage is reversible (temporary) under low to moderate intensity. In the case of temporary damage, a live cell heals itself after a short duration after withdrawing the electric field. Strong electric fields result in an irreversible effect, and ultimately, in cell death. Both types have been used in the different applications of food processing [25,27].

The fact that during PEF extraction the applied electric field causes electroporation of the cell membranes results in a more efficient diffusion of the bioactive components from the plant tissue. As a result, enhanced bioactive component recovery at ambient temperature and reduced extraction time are achieved. Moreover, PEF technique is effective with non-toxic green solvents used in a lesser quantity. The reduction of energy and solvents cost (ambient temperature, minimized extraction time, minimized quantity of cheaper solvents, evaporation of less quantity of solvent), environmental impact (effective in green solvents) and heat-sensitive compounds' degradation makes PEF a promising green alternative extraction technique, which can offset several of the disadvantages (longer extraction time, requirement of costly and high purity solvent, evaporation of the huge amount of solvent, low extraction selectivity and thermal decomposition of thermo labile compounds) of conventional extraction methods [28,29].

PEF technology is mostly evaluated in terms of its use in the food industry. According to the literature PEF can be a beneficial technique for the recovery of bioactive compounds and the reduction of microbial population. The potential of PEF technique for the enhanced extraction of high added value compounds was first explored by Brodelius et al. [30] and currently remains under constant investigation. PEF has been applied mostly to the valorization of fruit and vegetable industry wastes as an extraction method for the enhanced recovery of bioactive compounds, and as a pre-treatment prior to the extraction process to minimize the operational costs, reduce the environmental impact and achieve high yields of the desired compounds [26,31–40].

Regarding PEF application as an extraction method, it is worth mentioning that Pataro et al. [36] achieved enhanced extraction rate (27–37%), lycopene yields (12–18%) and antioxidant power (18.0–18.2%) from industrial derived tomato processing by-products applying a Pulsed Electric Field of 5 kV cm^{-1}. Corrales et al. [33] have investigated the combined effect of heat treatment (70 °C) with ultrasound (35 kHz), high hydrostatic pressure (600 MPa), and PEF (3 kV cm^{-1}) on grape pulp. According to their results, the application of PEF was more selective regarding the extraction of anthocyanins, and more effective, leading to a higher antioxidant activity. Finally, Turk et al. [38] succeeded in achieving an increase in polyphenols yield recovered from apple mash by applying an electric field strength of 450 V cm^{-1} for 10 milliseconds of specific energy less than 3 kJ kg^{-1}. Nevertheless, there are not many reports on the application of PEF as a primary, standalone extraction method of bioactive compounds from plants.

PEF application to the valorization of fruit and vegetable industry wastes as a pre-treatment method prior to extraction process is wider than its application as a primary extraction method, mentioned above [31,32,34,35,37,39,40]. In orange peel pre-treatment, PEF (7 kV cm^{-1}) treatment increased the total polyphenol extraction yield and the antioxidant activity of the extract up to 159% and 192%, respectively [34]. In another study, Yu et al. [39] studied the effectiveness of PEF treatment (0.2–10 kV cm^{-1}) as a new method of rapeseed stems and leaves valorization. The results showed that PEF treatment (5–20 kV cm^{-1}) increased proteins and polyphenols. In addition, PEF treatment at 5 kV cm^{-1} induced selective extraction of polyphenols for both rapeseed stems and leaves.

In this frame, the aim of the present work was to evaluate the potential of PEF as a primary, standalone extraction method in achieving increased recovery of phytochemicals (polyphenols) from the plants *C. sativa*, *R. canina* and *C. officinalis* using water as the extraction solvent, at room temperature and with a short extraction time. To improve the method efficiency, different PEF pulse duration times were studied. The evaluation of the extracts was determined by means of total and individual phenolics (quantified using the Folin–Ciocalteu method and high-performance liquid chromatography, respectively). To estimate the PEF effect, control extracts were prepared following the same procedure as for PEF extracts without the application of electric field.

2. Materials and Methods

2.1. Chemicals

Sodium carbonate anhydrous (99%) and gallic acid monohydrate were from Penta (Prague, Czech Republic). Phenolic standards (quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside, eriodictyol 7-*O*-rutinoside, hyperoside, isorhamnetin 3-*O*-rutinoside, catechin and ellagic and caffeic acid) used for chromatography were from Sigma Aldrich (St. Louis, MO, USA), while HPLC grade acetonitrile and formic acid (99%) were from Carlo Erba (Val de Reuil, France). Folin–Ciocalteu reagent was from Panreac (Barcelona, Spain).

2.2. Plant Material

Plant material of wild plants was a kind donation from Korres S.A. Natural Products (Oinofyta, Attiki, Greece). Leaves of *Castanea sativa*, fruits of *Rosa canina* and flowers of *Calendula officinalis* were collected during different seasons (spring and summer) of 2019 and air-dried (45% relative humidity) at a room temperature (24 °C) in the dark (1 day for the leaves of *C. sativa* and the flowers of *C. officinalis*, 1 week for the fruits of *R. canina*). The final water content reached after drying was 50% for *C. sativa*, and 30% for *R. canina* and *C. officinalis*. Then, they were ground (the rosa fruits without removing the stones) to particle sizes of about 0.5 mm using a blender (Camry, Poland) and stored in vacuum in the dark, at room temperature.

2.3. PEF Apparatus

The PEF system used was the static bench-scale system previously reported [41]. The equation $E = U\,d^{-1}$, where U is the applied voltage and d is the distance between the two electrodes (d = 1 cm) was used for the calculation of the applied electric field intensity.

2.4. PEF Extraction

The liquid to solid ratio for all extractions was maintained at 20:1 mL g^{-1} (1.25 g of plant material in 25 mL of double distilled water). The required amount of water was added to the ground plant material, the mixture was poured into the PEF treatment chamber and the PEF application started. After twenty minutes of extraction, the extracts were separated from the plant material by filtration. An infrared thermometer (GM300, Bentech, Shenzhen Jumaoyuan Science and Technology Co., Ltd., Shenzhen, China) was used to monitor the temperature of the treatment chamber contents before and after each extraction. The initial temperature of the extracts was 24 °C and was not significantly increased during PEF treatment ($\Delta t < 1$ °C).

PEF treated produced samples were compared to reference ones that were prepared in the same way but without the application of PEF. The same procedure was followed for all three plants. Extraction conditions for PEF and control extracts are summarized in Table 1. Pulse duration and period of 10 μsec and 1 msec, respectively, in different electric field intensities ranging from 1.2 to 2.0 kV cm^{-1}, were applied. Since each period (1 msec) corresponds to one pulse cycle, the number of cycles for an extraction time of 20 min equals to $N = 1.2 \times 10^6$. The total PEF treatment time, 12 sec in our study, derives from the product $t_{\text{Pulse}} \times N$. Specific energy can be calculated according to the following Equation (1) [42]:

$$W_{spec} = \sum_{0}^{N} \frac{N}{m} \int_{0}^{t_{Pulse}} U(t) \times I(t)dt \quad (1)$$

where, N is the total number of pulse cycles (dimensionless), m is the total weight of the sample (kg) poured into the PEF treatment chamber, t_{Pulse} is the duration of each pulse (sec), $U(t)$ is the output voltage (kV), and $I(t)$ the electric current (Ampere) applied to the sample. In the case of PEF static homogenous solid liquid extraction, where the same pulse type and pulse time duration are applied for each cycle, the values of $U(t)$ and $I(t)$ can be considered constant, and Equation (1) can be simplified to Equation (2):

$$W_{spec} = \frac{N}{m} \times U \times I \times t_{Pulse} \quad (2)$$

The total energy input, as calculated from Equation (2), ranged from 0.274 to 0.457 kJ kg^{-1}, depending on the applied electric field (specific energy) or 2.00×10^{-6} to 3.33×10^{-6} kWh (PEF energy) while specific energy per pulse (W_{spec}/N) ranged from 0.229 to 0.381 mJ kg^{-1} (Table 1).

Table 1. Extraction conditions for PEF and control extracts.

Extraction Condition	Extraction Time (min)	Temperature (°C)	Electric Field Intensity (kV cm^{-1})	Specific Energy (kJ kg^{-1})	Specific Energy Per Pulse (mJ kg^{-1})	PEF Energy (kWh)
PEF 1	20	24	2.0	0.457	0.381	3.33×10^{-6}
PEF 2	20	24	1.7	0.389	0.324	2.83×10^{-6}
PEF 3	20	24	1.4	0.320	0.267	2.33×10^{-6}
PEF 4	20	24	1.2	0.274	0.229	2.00×10^{-6}
Control	20	24	-	-	-	-

2.5. Total Polyphenol Content of Extracts

Total polyphenol content of the extracts was estimated based on a previously developed protocol [43]. The results were expressed as mg gallic acid equivalents (GAE) per gram of dry weight (dw). Yield in total polyphenols (Y_{TP}) was calculated according to the following Equation (3):

$$Y_{TP}\ (\text{mg GAE g}^{-1}\ \text{of dw}) = \frac{C_{TP} \times V}{w} \quad (3)$$

where, C_{TP} is the total polyphenol concentration of the extract (mg L^{-1}), V is the volume of the extraction medium (L) and w is the dry weight (g) of the plant material [44].

2.6. High-Performance Liquid Chromatography (HPLC)

A methodology previously reported was used [45]. In brief, a Shimadzu liquid Chromatograph (CBM-20A) and a Shimadzu detector (SPD-M20A) was used. A Phenomenex Luna C18(2) (100 Å, 5 µm, 4.6 × 250 mm) (Phenomenex, Inc., Torrance, CA, USA) retained at 40 °C, a flow rate was 1 mL min^{-1}, and an injection volume 20 µL were used. The mobile phases and the elution program used were described previously [45]. Detection was performed by scanning from 190 to 800 nm. Known standards were used for the identification and quantification of individual polyphenols. To identify specific metabolites, each one of them was compared to the corresponding standard, concerning its retention time and UV-Vis spectrum. Quantification was performed according to the equations of the standards calibration curves.

2.7. Statistical Analysis

Extractions as well as all determinations were carried out in triplicate. ANOVA test with SPSS (SPSS Inc., Chicago, IL, USA) software was used to evaluate statistical significance (at $p < 0.05$) of the differences between mean values.

3. Results and Discussion

During this study an alternative innovative technique (Pulsed Electric Field) [46,47] was applied for the extraction of valuable compounds (antioxidants) from plant material (*Rosa canina*, *Calendula officinalis* and *Castanea sativa*). Since this "green" technique does not use or produce heat (cold extraction) the decomposition of thermo labile compounds is avoided and extracts of high quality for use in fortified therapeutic decoctions, traditional beverages, or even alcoholic beverages are produced.

Various parameters are involved in PEF processing, the configuration of which can lead to different results. Those parameters are mainly summarized in the electric field strength, pulse shape, pulse duration and period, PEF treatment time, and specific energy [27,35,48,49]. Processing parameters can be optimized to achieve the release of only the desired compounds, keeping other compounds inside the cell. Under optimum conditions, the recovery factor is significantly increased with the minimum extraction time [26]. From the aforementioned factors, electric field strength and treatment time (t = number of pulses $\times$ pulse duration) are central processing factors, and their increase is considered to enhance the processing efficiency of PEF [48,50].

Electric field intensity affects the electroporation of the cell membrane depending on the dimension of the target cell. The frequency and pulse duration of the applied high voltage pulses, also determine the magnitude of the membrane's disturbance, affecting the cell efficiency to disintegrate and expel intracellular substances. According to recent studies, pulses of micro or milliseconds and electric intensities $E > 1500$ V cm^{-1}, are the more suitable values to achieve an effective extraction of bioactive compounds with PEF [26,48].

During this study different, relatively high, electric field intensities up to 2 kV cm^{-1} and short pulses of 10 μsec were applied to determine the possibility of application of the PEF technique to the production of plant extracts, using pure water as the extraction solvent. Leaves of *C. sativa*, fruits of *R. canina* and flowers of *C. officinalis* were selected for their medicinal properties as raw material [12–14]. The extracts obtained were evaluated concerning their content in total polyphenols and their identified polyphenolic compounds.

3.1. Total Polyphenol Content of Extracts

The content of aqueous plant extracts in polyphenols was estimated via the Folin–Ciocalteu method. PEF technique appeared to be effective for the enhanced extraction of polyphenols, using water as the extraction solvent, for all the plant materials examined. Results regarding the effect of electric field intensity to the extraction yield were similar for the three plants (Figures 1–3). The higher recovery in total polyphenols was reached by the application of moderate to high electric field, up to 1.4 kV cm^{-1} (conditions PEF 3 and PEF 4). More specifically, the significantly ($p < 0.05$) higher increase in the recovery, 63.79%, was achieved in the case of *R. canina* fruits, applying Pulsed Electric Field of 1.4 kV cm^{-1}, condition PEF 3. Condition PEF 4 appeared to be the most effective (significant at $p < 0.05$) for *C. officinalis* and *C. sativa*, resulting in increases of 55.02% and 48.41%, respectively. Electric field intensities of 1.7 and 2.0 kV cm^{-1} appeared also to be effectual, though led to lower increases, not exceeding 35%.

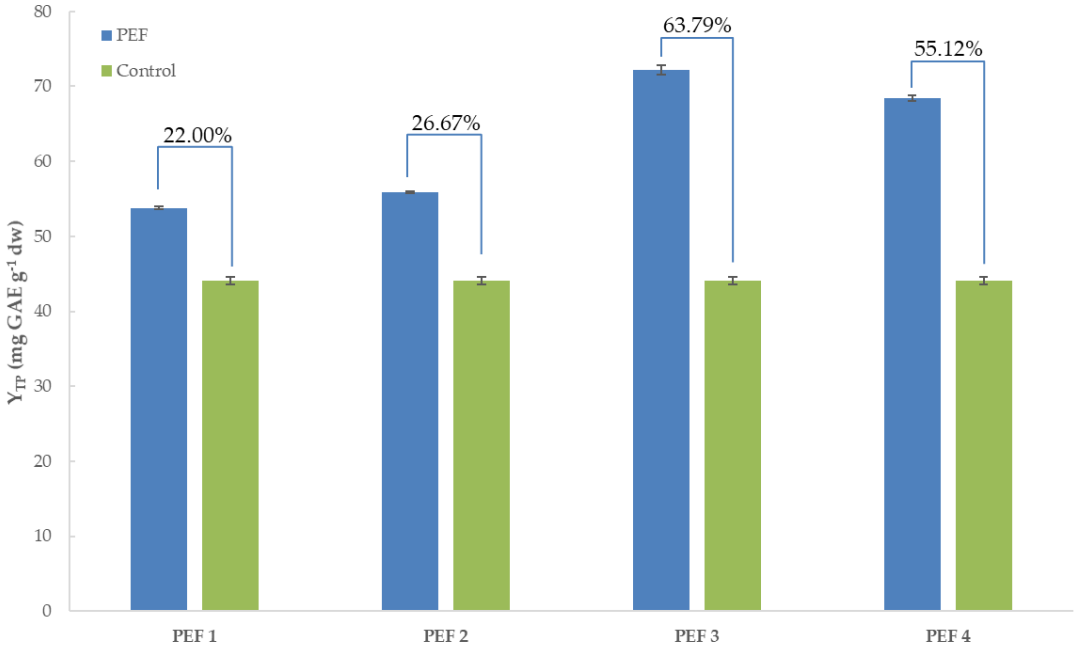

Figure 1. Total phenolics, Y_{TP}, for *Rosa canina* fruits PEF samples acquired in different electric field intensities, compared to control sample.

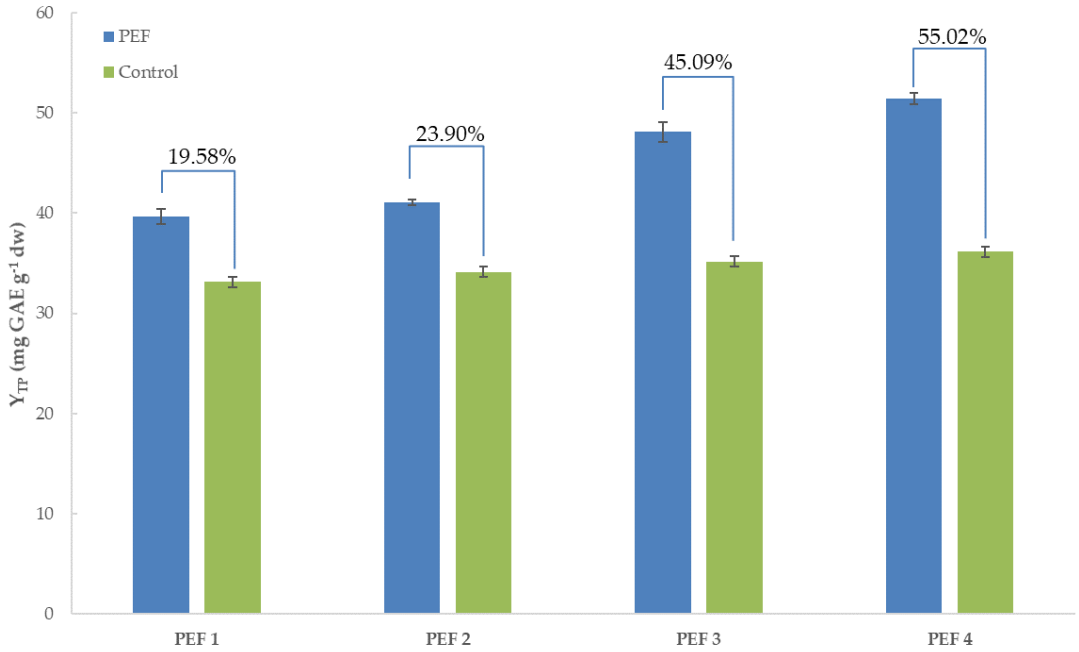

Figure 2. Total phenolics, Y_{TP}, for *Calendula officinalis* flowers PEF samples, in different PEF electric field intensities, compared to control sample.

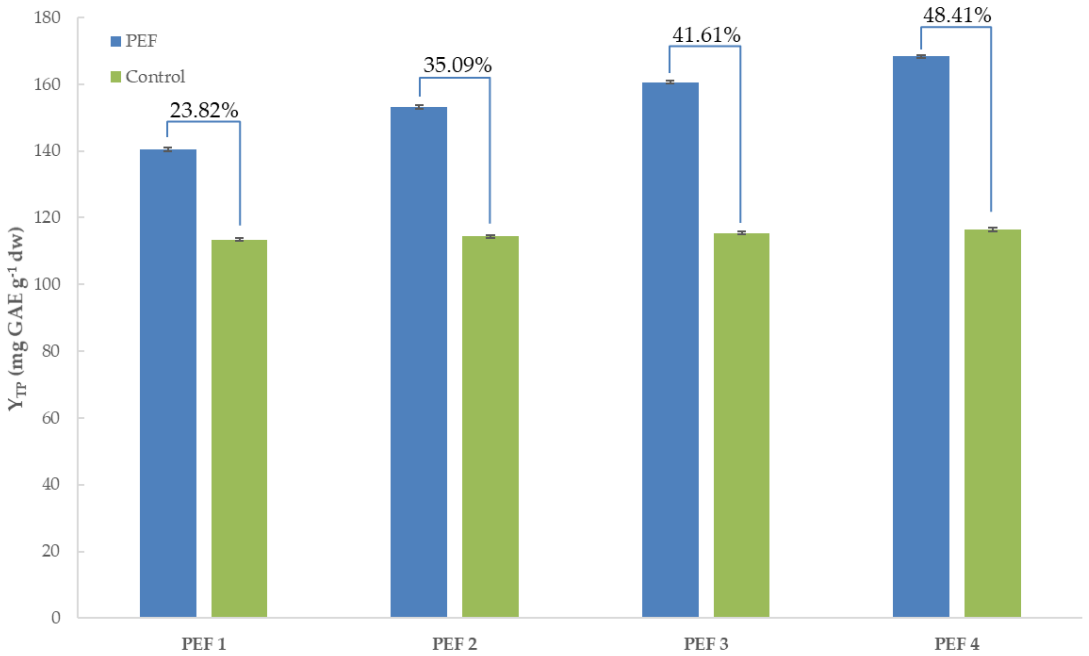

Figure 3. Total phenolics, Y_{TP}, for *Castanea sativa* leaves PEF samples in different electric field intensities, compared to control samples.

3.2. Composition of Polyphenolic Compounds

Our results, in relation to the composition of examined plants in polyphenols, are presented in Tables 2–4 and are in line with the above relevant literature [15,16,18,19]. Specifically, in *R. canina* fruits, quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside, eriodictyol 7-*O*-rutinoside and catechin in the amounts 0.012 ± 0.002, 0.121 ± 0.005, 0.032 ± 0.005 and 0.273 ± 0.017 mg g^{-1} dw, respectively, were identified. Quercetin 3-*O*-rutinoside has indeed been mentioned as a basic glycoside of *R. canina* fruits, while eriodictyol 7-*O*-rutinoside and quercetin 3-*O*-glucoside were mentioned to be contained in smaller quantities [18,19]. Analysis of *C. sativa* leaf extracts resulted in the determination of the polyphenols quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside, hyperoside (quercetin-3-*O*-galactoside) and ellagic acid, while analysis of *C. officinalis* flowers extract revealed the existence of quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside, isorhamnetin 3-*O*-rutinoside and caffeic acid.

Table 2. Identified polyphenolic compounds of *Rosa canina* (fruits) extracts.

Rosa canina								
Extraction Condition	**Individual Polyphenol Content, mg g^{-1} dw (Mean Values of Three Replicates, RSD [1] = 0.05–1%)**							
	Quercetin 3-*O*-glucoside		**Quercetin 3-*O*-rutinoside**		**Eriodictyol 7-*O*-rutinoside**		**Catechin**	
	Average [2]	**Increase [3] (%)**	**Average**	**Increase (%)**	**Average**	**Increase (%)**	**Average**	**Increase (%)**
PEF 1	0.015 ± 0.002 ^{a}	25 ± 3 ^{a}	0.146 ± 0.007 ^{b}	21 ± 0 ^{a}	0.039 ± 0.003 ^{a}	21 ± 8 ^{a}	0.328 ± 0.024 ^{b}	20 ± 1 ^{a}
PEF 2	0.015 ± 0.003 ^{a}	24 ± 5 ^{a}	0.149 ± 0.006 ^{b}	23 ± 1 ^{b}	0.040 ± 0.005 ^{a}	24 ± 2 ^{a}	0.344 ± 0.021 ^{b}	26 ± 0 ^{b}
PEF 3	0.021 ± 0.002 ^{b}	74 ± 9 ^{b}	0.206 ± 0.009 ^{d}	71 ± 0 ^{d}	0.059 ± 0.006 ^{b}	84 ± 9 ^{b}	0.450 ± 0.032 ^{c}	65 ± 2 ^{d}
PEF 4	0.020 ± 0.002 ^{b}	66 ± 8 ^{b}	0.188 ± 0.008 ^{c}	55 ± 0 ^{c}	0.054 ± 0.004 ^{b}	69 ± 13 ^{b}	0.420 ± 0.016 ^{c}	54 ± 4 ^{c}
Control	0.012 ± 0.002 ^{a}	-	0.121 ± 0.005 ^{a}	-	0.032 ± 0.005 ^{a}	-	0.273 ± 0.017 ^{a}	-

[1] RSD = Relative standard deviation; [2] Values are expressed as the mean values (±SD) of triplicate determinations and means within each column with different superscript letters are significantly ($p < 0.05$) different; [3] Increase (%) = ($C_{PEF} - C_{Control}/C_{Control}$) × 100.

Concerning PEF effect and the utilization of different electric field intensities, there is a trend, as in total polyphenols determination, that indicates conditions PEF 3 and PEF 4 as more effective. More precisely, in the case of *R. canina* fruits (Table 2) the application

of condition PEF 3 led to an increase of the basic glycoside, quercetin 3-*O*-rutinoside of 71%. Corresponding increases for eriodictyol 7-*O*-rutinoside, quercetin 3-*O*-glucoside and catechin, were also significant ($p < 0.05$), reaching 84%. Condition PEF 4 gave relatively lower recoveries, resulting in an increase of 69% (in eriodictyol 7-*O*-rutinoside) while conditions PEF 1 and PEF 2 did not lead to corresponding increases.

In *C. officinalis* flowers (Table 3), the optimum PEF condition appeared to be condition PEF 4. Applying pulse electric field of 1.2 kV cm^{-1}, an increase of 73% in isorhamnetin 3-*O*-rutinoside, 66% in caffeic acid, 65% in quercetin 3-*O*-rutinoside and 63% in quercetin 3-*O*-glucoside was achieved. Corresponding recoveries achieved with condition PEF 3, 1.4 kV cm^{-1}, were 3–6% lower, while conditions PEF 1 and PEF 2 of higher intensities, again led to significant lower recoveries.

Table 3. Identified polyphenolic compounds of *Calendula officinalis* (flowers) extracts.

Calendula officinalis								
Extraction Condition	**Individual Polyphenol Content, mg g^{-1} dw (Mean Values of Three Replicates, RSD [1] = 0.05–1%)**							
	Quercetin 3-*O*-glucoside		**Quercetin 3-*O*-rutinoside**		**Isorhamnetin 3-*O*-rutinoside**		**Caffeic Acid**	
	Average [2]	**Increase [3] (%)**	**Average**	**Increase (%)**	**Average**	**Increase (%)**	**Average**	**Increase (%)**
PEF 1	1.640 ± 0.075 ^{b}	26 ± 11 ^{a}	2.847 ± 0.279 ^{a}	22 ± 1 ^{a}	9.599 ± 0.657 ^{b}	22 ± 3 ^{a}	0.782 ± 0.074 ^{a}	22 ± 14 ^{a}
PEF 2	1.706 ± 0.189 ^{b}	30 ± 3 ^{a}	2.918 ± 0.340 ^{a}	25 ± 1 ^{b}	10.071 ± 0.400 ^{b}	28 ± 1 ^{b}	0.822 ± 0.099 ^{a}	28 ± 11 ^{a}
PEF 3	2.073 ± 0.133 ^{c}	59 ± 11 ^{b}	3.781 ± 0.359 ^{b}	62 ± 2 ^{c}	13.376 ± 0.479 ^{c}	70 ± 2 ^{c}	1.030 ± 0.096 ^{b}	61 ± 19 ^{b}
PEF 4	2.125 ± 0.140 ^{c}	63 ± 11 ^{b}	3.851 ± 0.417 ^{b}	65 ± 0 ^{d}	13.612 ± 0.633 ^{c}	73 ± 0 ^{d}	1.069 ± 0.127 ^{b}	66 ± 15 ^{b}
Control	1.312 ± 0.174 ^{a}	-	2.334 ± 0.253 ^{a}	-	7.868 ± 0.377 ^{a}	-	0.652 ± 0.134 ^{a}	-

[1] RSD = Relative standard deviation; [2] Values are expressed as the mean values (±SD) of triplicate determinations and means within each column with different superscript letters are significantly ($p < 0.05$) different; [3] Increase (%) = $(C_{PEF} - C_{Control}/C_{Control}) \times 100$.

Additionally, in the case of *C. sativa* (Table 4), condition PEF 4 led to the higher recoveries, followed by condition PEF 3. The increases achieved via application of condition PEF 4 reached 82% for quercetin 3-*O*-glucoside, 72% for hyperoside and ellagic acid and 62% for quercetin 3-*O*-rutinoside, while corresponding percentages for condition PEF 3 ranged from to 50% to 70%.

Table 4. Identified polyphenolic compounds of *Castanea sativa* (leaves) extracts.

Castanea sativa								
Extraction Condition	**Individual Polyphenol Content, mg g^{-1} dw (Mean Values of Three Replicates, RSD [1] = 0.05–1%)**							
	Quercetin 3-*O*-glucoside		**Quercetin 3-*O*-rutinoside**		**Hyperoside**		**Ellagic Acid**	
	Average [2]	**Increase [3] (%)**	**Average**	**Increase (%)**	**Average**	**Increase (%)**	**Average**	**Increase (%)**
PEF 1	1.499 ± 0.117 ^{b}	30 ± 1 ^{a}	6.869 ± 0.357 ^{b}	20 ± 2 ^{a}	4.269 ± 0.256 ^{b}	25 ± 0 ^{a}	3.444 ± 0.182 ^{b}	27 ± 4 ^{a}
PEF 2	1.545 ± 0.110 ^{b}	34 ± 2 ^{b}	6.926 ± 0.375 ^{b}	21 ± 2 ^{a}	4.371 ± 0.253 ^{b}	28 ± 0 ^{b}	3.526 ± 0.199 ^{b}	30 ± 3 ^{a}
PEF 3	1.960 ± 0.055 ^{c}	71 ± 10 ^{c}	8.700 ± 0.315 ^{c}	52 ± 5 ^{b}	5.396 ± 0.336 ^{c}	58 ± 0 ^{c}	4.068 ± 0.218 ^{c}	50 ± 4 ^{b}
PEF 4	2.098 ± 0.156 ^{c}	82 ± 2 ^{c}	9.273 ± 0.309 ^{c}	62 ± 6 ^{b}	5.874 ± 0.362 ^{c}	72 ± 0 ^{d}	4.665 ± 0.298 ^{d}	72 ± 3 ^{c}
Control	1.153 ± 0.098 ^{a}	-	5.724 ± 0.397 ^{a}	-	3.415 ± 0.210 ^{a}	-	2.712 ± 0.224 ^{a}	-

[1] RSD = Relative standard deviation; [2] Values are expressed as the mean values (±SD) of triplicate determinations and means within each column with different superscript letters are significantly ($p < 0.05$) different; [3] Increase (%) = $(C_{PEF} - C_{Control}/C_{Control}) \times 100$.

According to the results, PEF does have the potential to increase the extractability of polyphenols from the plants *C. sativa*, *R. canina* and *C. officinalis* using pure water as the extraction solvent. Moderate electric field intensities, 1.2 to 1.4 kV cm^{-1}, and short pulses of 10 μsec are proposed in order to achieve higher extraction efficiency.

It can be concluded that PEF is a very promising technique in the field of bioactive compounds extraction from plants that could offset several of the disadvantages of conven-

tional extraction methods. This denotes the possibility of a large-scale application of the method. Moreover, it indicates the environmentally friendly production of high-quality extracts that can be used in sweetened or unsweetened beverages, drinks, and snacks. The degradation of thermosensitive compounds and the cost of energy are reduced by using ambient temperature and a short extraction time. The use of water as an extraction solvent simplifies the entire process (production of edible extracts without the requirement for solvent evaporation), as well as lowers the costs and reduces the environmental impact (water is a cheap and non-toxic solvent).

During our previous work, polyphenols were successfully extracted from leaves of *Moringa oleifera* applying a Pulsed Electric Field of 7 kV cm^{-1}, with pulse duration and pulse interval 20 msec and 100 μsec, respectively [28]. Ntourtoglou et al. [51] achieved an increase of 20% in the extraction rate of the alpha acids of bitter hop using PEF. Tsapou et al. [52] applied a Pulsed Electric Field (PEF) to beer wort, enriched with flax seeds, to reach phenolic flavor enhancement in beer, succeeding in 4-vinylguaiacol (target compound) production efficiency up to 120% (mg L^{-1}). Finally, Lakka et al. [41] successfully extracted polyphenols from the aerial parts of *Sideritis scardica*, tepals of *Crocus sativus*, and fruits of *Vitis vinifera* using a PEF of 1.2–1.4 kV cm^{-1}.

PEF processing parameters including pulse shape, pulse duration, pulse frequency and treatment time, could be further optimized in a future work. This way a full evaluation of PEF potential as a "green", selective, cold extraction technique of high added value compounds that can be of further use from all food, cosmetic and pharmaceutical industries interested in the production of bio-functional foods/dietary supplements, cosmetics, and new drugs, could be achieved.

4. Conclusions

The aim of this study was to determine whether the PEF technique could be used to produce plant extracts with high concentrations of bioactive compounds using water as the extraction medium. To optimize the procedure, various different electric field intensities were evaluated, while short pulses of 10 μsec were delivered over a period of 1 msec with a total extraction time of 20 min. In comparison to the control samples, PEF treatment of *Rosa canina* fruits, *Calendula officinalis* flowers and *Castanea sativa* leaves resulted in extracts with increased polyphenol content. PEF application showed that highest increase in the yield of total polyphenols was from *R. canina* fruits (63.79%). In the same plant material, the corresponding increase in individual polyphenols reached 84% for eriodictyol 7-*O*-rutinoside. The concentration of the extracted polyphenols appears to depend on the electric field intensity (significant at $p < 0.05$). It can be concluded that PEF is a very promising "green" technique for extracting bioactive compounds from plants that can be used in food (as herbal teas, traditional beverages, or even alcoholic beverages), cosmetics, and the pharmaceutical industry. More research is needed to fully understand the application of PEF, which could lead to more efficient extracts that can be used to fortify medicinal herbal teas, traditional beverages, or even alcoholic beverages. In particular, because *R. canina* fruits are of high industrial importance, a much more detailed study (including extraction optimization) should be carried out.

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