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# Pulsed electric field–assisted green extraction of cocoa pod husk: enhancement of antioxidant and tyrosinase inhibitory activities

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## ABSTRACT

Cocoa pod husk (CPH), a major by-product of cocoa processing, is an abundant yet underutilized biomass rich in bioactive compounds with promising applications in food, cosmetic, and cosmeceutical industries. This study explored the potential of pulsed electric field (PEF)–assisted green extraction combined with water–ethanol solvent systems to enhance the recovery of bioactive constituents from CPH. The influence of solvent polarity and PEF pulse numbers on extraction performance were systematically evaluated. Extractions were carried out using water and water–ethanol mixtures at ratios of 75:25, 50:50, and 25:75 (v/v), under a PEF treatment at an electric field strength of 6 kV/cm with pulse numbers ranging from 1,000 to 5,000. The resulting extracts were characterized in terms of total phenolic, flavonoid, and tannin contents, reducing sugar levels, antioxidant capacity (DPPH and FRAP assays), and tyrosinase inhibitory activity. The findings revealed that extraction efficiency was strongly dependent on both solvent composition and PEF parameters. Hydroethanolic solvents with intermediate polarity, particularly 50–75% ethanol, significantly enhanced the extraction of phenolics, flavonoids, and tannins compared with water or higher ethanol concentrations. The application of moderate PEF pulse numbers (1,000–3,000 pulses) effectively promoted cell membrane permeabilization, thereby improving mass transfer and facilitating the release of intracellular bioactive compounds. Antioxidant activities determined by DPPH and FRAP assays showed consistent trends, with the highest antioxidant capacity observed under hydroethanolic extraction conditions combined with moderate PEF treatment. Notably, tyrosinase inhibitory activity was maximized in water and hydroethanolic extracts, suggesting that enzyme inhibition is primarily governed by the selective extraction and synergistic interactions of specific phenolic constituents rather than total phenolic content alone. This study demonstrates that PEF-assisted extraction using environmentally friendly water–ethanol solvents is an efficient and sustainable strategy for the valorization of cocoa pod husk, highlighting its strong potential as a functional ingredient for food, cosmetic, and cosmeceutical applications.

**Keywords:** Pulse Electric Field, Cocoa Pod Husk, antioxidant, tyrosinase inhibitor, SDG 12, BCG model

## INTRODUCTION

Cocoa husk is generated as a major by-product of cocoa processing and is commonly regarded as waste, thereby posing challenges for environmental management. However, cocoa husk represents a valuable source of bioactive constituents, including polyphenols, dietary fiber, and phytosterol compounds. These components can be recovered and subsequently valorized for use in food, nutraceutical, and health-related products, in alignment with Sustainable Development Goal 12, which emphasizes the sustainable management and efficient use of natural resources, as well as the Bio-Circular-Green (BCG) economy model.

Pulsed electric field (PEF) technology is a non-thermal and environmentally friendly extraction approach that employs short, high-voltage electric pulses to enhance the release of bioactive compounds from plant tissues. In this technique, plant materials are subjected to electric fields in the microsecond-to-millisecond range, typically at field strengths of 0.1–50 kV/cm, between a pair of electrodes. The applied electric pulses induce electroporation of cell membranes, resulting in the formation of transient or permanent pores that increase membrane permeability and facilitate the mass transfer of intracellular compounds.

Pulsed electric field (PEF) treatment has been extensively applied to improve the extraction

efficiency of bioactive compounds from a variety of food matrices, including sugar beet juice, cocoa bean shells, and coffee silverskin (Barbosa-Pereira et al., 2018). Previous studies have reported that extracts obtained using PEF exhibit higher antioxidant activity than those produced by conventional extraction methods. This enhancement has been attributed to PEF-induced permeabilization of cocoa pod husk (CPH) cell membranes, which promotes the release of intracellular bioactive constituents. Consequently, the increased availability of these compounds contributes to the enhanced antiaging enzyme activity observed in PEF-treated extracts.

The objective of this study was to investigate the extraction of secondary metabolites and enzyme-related activities from cocoa pod husk (CPH) using pulsed electric field (PEF) treatment. The effects of PEF on key bioactive constituents, including total phenolic, flavonoid, and tannin contents, as well as antioxidant activity, were systematically evaluated as the primary indicators of extraction efficiency. The findings of this study are expected to provide a scientific basis for further research and the development of cosmetic applications utilizing bioactive compounds derived from CPH.

## MATERIALS AND METHODS

### *Materials*

The cocoa pod husk (CPH) variety Chumphon 1 was obtained as a gift from Mr. Soravit Panpinij, owner of Rai Chuan Fun Farm, located in Phichai Subdistrict, Mueang Lampang District, Lampang Province, Thailand (52000). The CPH samples were collected between January and May 2025. Species identification was confirmed by a qualified herbalist affiliated with Rajamangala University of Technology Lanna (RMUTL).

### *Methods*

### *Sample Preparation*

Upon arrival, 100 g of intact cocoa pods were washed with tap water followed by distilled water to remove surface contaminants. The cleaned samples were subsequently ground using an electric blender to obtain a homogeneous paste. The resultingpaste was mixed with extraction solvents, including water, ethanol, and water–ethanol mixtures at ratios of 75:25, 50:50, and 25:75 (v/v). The mixture was then passed through a 20-mesh sieve to obtain a fine, uniform material, which was stored at −20 °C until further use.

### *Pulsed Electric Field Treatment*

Pulsed electric field (PEF) treatment was conducted in a batch system following the method described by Salee et al. (2022), with minor modifications. Briefly, the electric field generator was operated at 6 kV using a 220 VAC, 50 Hz power supply with a maximum power output of 500 W. Electrical energy was stored in a 0.1 µF capacitor charged to 6 kV prior to discharge through a rotating spark gap switch, generating high-voltage electric pulses delivered to the treatment chamber.

The extraction chamber consisted of a coaxial cylindrical PEF configuration with inner and outer electrode diameters of 20 mm and 60 mm, respectively. After soaking the cocoa pod husk (CPH) paste in the selected solvent for 10 min, the sample was loaded into the treatment chamber. PEF-assisted aqueous extraction (PEF-AWE) was performed at an electric field strength of 6 kV/cm, with the number of pulses ranging from 1,000 to 5,000, a constant pulse duration of 1 µs, and a pulse repetition frequency of 5 Hz. In all experiments, the initial sample temperature was maintained at 25.0 ± 1.3 °C, and the treated samples were subsequently stored at 4 °C prior to further analysis.

### *Determination of total phenolic content*

According to Sassa-deepaeng et al. (2023), the Folin-Ciocalteu (FC) colorimetric method was used to estimate the total phenolic content (TPC). After mixing 20 µL of the extract at different concentrations with 100 µL of FC reagent (Merck, Darmstadt, Germany) and 1,980 µL of deionized water, the mixture was incubated for five minutes at room temperature. The mixture was then incubated for an additional 60 minutes at room temperature in the dark after 300 µL of a 7% sodium carbonate solution (Qrec, Auckland, New Zealand) was added. Utilizing a Metash UV-5200 spectrophotometer equipped with UV-Professional analysis software, absorbance was measured at 765 nm. The study of the total phenolic content (TPC) was carried out three times. Several concentrations of gallic acid standards (Bio Basic Inc., Ontario, Canada) were used to create a calibration curve. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry sample weight.

### Determination of total flavonoid content

Total flavonoid content (TFC) was also estimated using Sassa-deepaeng et al.'s technique (2023). After making a combination with 20 µL of extract and 380 µL of deionized water, 100 µL of a 5% sodium nitrite solution (Univar, Ajax Finechem, Australia) was added. The mixture was incubated for 5 minutes, then 100 µL of a 10% aluminum chloride solution (Univar, Ajax Finechem, Australia) was added. It was then allowed to stand at room temperature for 6 minutes. The 400 µL solution of 1M sodium hydroxide (Labscan, Bangkok, Thailand) was then added. Following a 15-minute dark incubation period at room temperature, absorbance at 415 nm was measured. The TFC was calculated using a calibration curve prepared with quercetin (Sigma–Aldrich, Germany) at different concentrations and expressed as milligrams of quercetin equivalent (QE) per gram of dry weight.

### Determination of total tannin content

According to Sassa-deepaeng et al. (2023), the total tannin content (TTC) was also calculated by vigorously mixing 250 µL of extract with 450 µL of 1% vanillin (Merck KGaA, Darmstadt, Germany) reagent. 300 µL of concentrated hydrochloric acid (Labscan, Bangkok, Thailand) was added after the mixture had been incubated for five minutes, and it was then allowed to sit at room temperature for another half hour. A red tint appeared in the solution. At 500 nm, absorbance was then measured. The TTC was reported as milligrams of epigallocatechin gallate equivalent (EGCGE) per gram of dry sample weight and was computed using a calibration curve made with epigallocatechin gallate (Myskinrecipes, Bangkok, Thailand) at various doses.

### Determination of reducing sugar content

The reducing sugar content was determined using the dinitrosalicylic acid (DNS) method, as described by Teanprapakun et al. (2025), with slight modifications. The DNS reagent (Fluka Chemie GmbH, Buchs, Switzerland) was prepared by dissolving 2.5 g of DNS in 100 mL of 1 M sodium hydroxide, followed by the addition of a hot sodium potassium tartrate solution (75 g dissolved in 125 mL of distilled water). The resulting mixture was diluted to a final volume of 500 mL with distilled water. For the assay, 0.9 mL of DNS reagent was mixed with 0.1 mL of the aqueous plant extract in a 1.5 mL microcentrifuge tube. The mixture was then heated in a boiling water bath for 5 minutes, leading to the development of a red-brown color. After cooling, absorbance was measured at 540 nm using a spectrophotometer. Reducing sugar content was quantified using a standard calibration curve generated with 2 mM glucose solution (Univar, Ajax Finechem, Australia) and expressed as milligrams per gram of dry sample weight.

### Determination of Saponin content

The Singh et al. (2019) method was used to determine the saponin content. Two reagents were mixed in equal amounts: a 0.5% para-anisaldehyde (Myskinrecipes, Bangkok, Thailand) solution in ethyl acetate (Labscan, Bangkok, Thailand) and a 50% sulfuric acid (Labscan, Bangkok, Thailand) solution in ethyl acetate (Labscan, Bangkok, Thailand). Two milliliters of ethyl acetate were used to dissolve the aqueous extracts of the standard and the tentative plant sample. Reagents A and B were then added, one milliliter each. After mixing the solution, it was incubated in a dry bath incubator (Major Science Co., Ltd., Taoyuan, Taiwan) for ten minutes at 60°C. The absorbance at 430 nm was measured after cooling to room temperature. Using a calibration curve made using quillaja bark saponin (Sigma Aldrich, Saint Louis, MO, USA) at various doses, the saponin content was determined and represented as milligrams of saponin equivalent (SE) per gram of dry sample weight.

### Determination of tyrosinase

Anti-tyrosinase activity was evaluated according to the method described by Yodthong et al. (2020), with minor modifications. Briefly, the standard inhibitor and test extracts (400 µg/mL) were initially dissolved in ethanol (Liquor Distillery Excise Department, Bangkhla, Thailand) and subsequently diluted with 50 mM sodium phosphate buffer (pH 6.6). The extracts were first screened at a concentration of 400 µg/mL to assess their inhibitory activity against tyrosinase. Samples exhibiting inhibitory activity were then selected for further analysis at various concentrations ranging from 3.125 to 800 µg/mL. Kojic acid was used as the positive control.

For the assay, 70 µL of each extract solution was mixed with 30 µL of tyrosinase solution (333 U/mL) in a 96-well microplate (Sterilin Limited, UK)

and incubated for 5 min at ambient temperature. Subsequently, 110 µL of substrate solution containing 4 mM L-3,4-dihydroxyphenylalanine (L-DOPA) was added to each well. The reaction mixtures were incubated for an additional 30 min at ambient temperature in the dark. The formation of dopachrome was measured by monitoring absorbance at 492 nm using a microplate reader (BIOBASE-EL10, Biobase Biodustry (Shandong) Co., Ltd., China). The percent inhibition of tyrosinase was calculated as the following equation:

$$\%\text{Tyrosinase inhibition} = [(A-B)-(C-D)/(A-B)] \times 100$$

Whereas,
A = absorbance of blank solution with tyrosinase
B = absorbance of blank solution without tyrosinase
C = absorbance of sample solution with tyrosinase
D = absorbance of sample solution without tyrosinase

### *Determination of Antioxidant*

### *DPPH Radical-Scavenging Activity Assay*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was evaluated using a modified method described by Sassa-deepaeng et al. (2019). Briefly, a DPPH solution (Sigma–Aldrich, Steinheim, Germany) was prepared in absolute ethanol to obtain an initial absorbance of 0.8 ± 0.1 at 517 nm. Aliquots of 100 µL of sample solutions at various concentrations (125, 250, 500, and 1,000 µg/mL) were mixed with 900 µL of the DPPH radical solution. The reaction mixtures were incubated at ambient temperature for 30 min in the dark.

The antioxidant activity of the samples was determined based on their ability to donate electrons to DPPH radicals, resulting in a decrease in the purple coloration of the solution. Absorbance was measured at 517 nm against a blank using a V-1200 UV–Vis spectrophotometer (Dshing Instrument Co., Ltd., China) equipped with UV-Professional Analysis software. The percentage of inhibition of antioxidant was calculated using the equation:

$$\%\text{ inhibition} = [(Ac-As)/Ac] \times 100$$

Where $A_c$ was the absorbance of the control and $A_s$ was the absorbance of the reaction mixture.The linear curves were constructed by plotting the percentage of inhibition against the concentration in µg/mL ($R^2$= 0.99).

### *Ferric reducing antioxidant power assay*

The ferric reducing antioxidant power (FRAP) assay was conducted in triplicate using 96-well microplates following the protocol described by Pranamornkith et al. (2022). The FRAP working solution was prepared by mixing 0.3 M acetate buffer (pH 3.6), 0.01 M hydrochloric acid (Labscan, Thailand), and 0.02 M ferric chloride hexahydrate (Merck, Germany) in a volumetric ratio of 10:1:1, respectively. A volume of 20 µL of appropriately diluted sample was added to 280 µL of the FRAP working solution in each well. The reaction mixture was incubated at ambient temperature for 30 minutes in the dark to allow the formation of the $Fe^{2+}$–tripyridyltriazine (TPTZ) complex. Absorbance was then measured at 630 nm using a microplate reader (Biobase EL-10A, China). The antioxidant capacity of the samples was expressed as micrograms of gallic acid equivalents (GE) per gram of dry weight (DW).

### *Statistical analysis*

Data analysis was performed using the Analysis ToolPak add-in in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). One-way analysis of variance (ANOVA) was conducted to assess differences among treatment groups. Where significant differences were detected, the least significant difference (LSD) test was applied for post hoc comparisons. Statistical significance was defined at $p < 0.05$.

## RESULTS AND DISCUSSION

Pulsed electric field (PEF) treatment played a critical role in facilitating the release of endogenous bioactive compounds from cocoa pod husk (CPH). To optimize the extraction of these compounds, CPH paste was subjected to PEF-assisted extraction using green solvents of varying polarity, specifically water–ethanol mixtures at volumetric ratios of 75:25, 50:50, and 25:75, in combination with an electric field strength of 6 kV/cm and pulse numbers of 1,000, 3,000, and 5,000. Consistent with the principles of solubility, the extraction efficiency of bioactive compounds was influenced by solvent polarity. The total phenolic content obtained under these extraction conditions is presented in Figure 1.

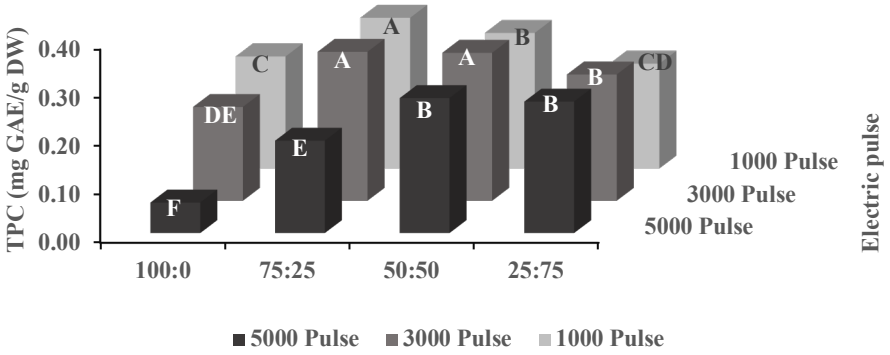


**Figure 1.** The effect of different solvent mixture ratios on total phenolic content. The different letters indicate significant difference ($p < 0.05$).

The extraction using a water–ethanol solvent at a volumetric ratio of 100:0 was not conducted following PEF treatment because the sample underwent sudden thermal damage and burning immediately after the application of the electric field.

As shown in Figure 1, the water–ethanol solvent mixture at a ratio of 75:25 (v/v) yielded the highest total phenolic content when PEF pulse numbers of 1,000 and 3,000 were applied. In contrast, a decreasing trend in phenolic yield was observed with increasing ethanol proportions in the extraction solvent. This behavior may be attributed to the structural characteristics of phenolic compounds, which contain both polar hydroxyl (–OH) groups and nonpolar aromatic rings, rendering them amphipathic in nature.

The water–ethanol mixture at a 75:25 ratio provides an intermediate polarity that is well suited for the solubilization of phenolic compounds. The presence of water facilitates hydrogen bond formation with hydroxyl groups, while the ethyl moiety ($–CH_2CH_3$) of ethanol enhances the solubility of the aromatic structures. Consequently, this solvent composition aligns with the principle of "like dissolves like," thereby resulting in improved extraction efficiency of phenolic compounds as indicated by the results of Athanasiadis et al. (2022) and Barbosa-Pereira et al. (2018)

The total flavonoid content obtained under these extraction conditions was presented in Figure 2.

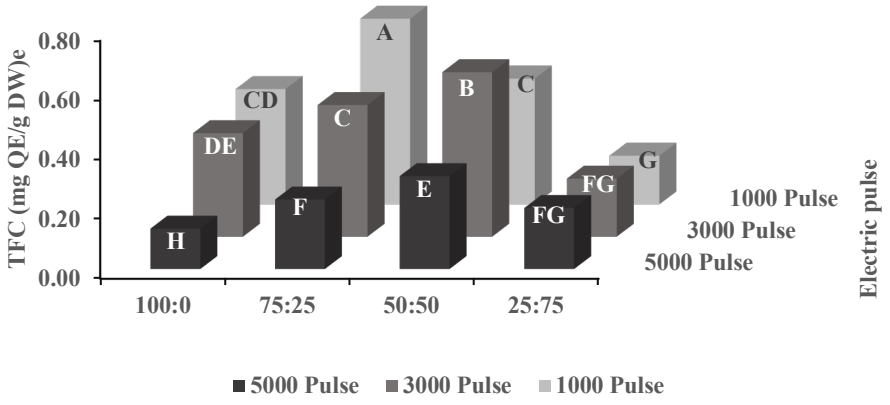


**Figure 2.** The effect of different solvent mixture ratios on total flavonoid content. The different letters indicate significant difference ($p < 0.05$).

As indicated in figure 2, the water–ethanol solvent mixture at a ratio of 75:25 (v/v) also yielded the highest total flavonoid content when PEF pulse numbers of 1,000 was applied. In contrast, flavonoid yield exhibited a decreasing trend with increasing ethanol proportions in the extraction solvent. This trend is consistent with the structural characteristics of flavonoids, a subclass of phenolic compounds, which influence their solubility behavior under different solvent polarities.

Previous studies have reported that ethanol at concentrations of 60–80% is an effective solvent for the extraction of flavonoids and phenolic compounds from various plant materials, yielding high extraction efficiency and antioxidant activity (Wang et al., 2022; El Mannoubi, 2023). Consistent with these findings, the present results demonstrate that 75% ethanol when PEF pulse numbers of 1,000 was the most suitable solvent for flavonoid extraction, providing an optimal balance between solubility and solvent penetration. Nevertheless, the water–ethanol solvent mixture at a ratio of 50:50 (v/v) also produced high total flavonoid contents when PEF pulse numbers of 3,000 or 5,000 were applied. This outcome is associated with the increased extent of cell membrane permeabilization induced by higher pulse numbers, which enhances mass transfer of intracellular flavonoids into the solvent. Repeated pulsing may further facilitate solvent penetration and weaken cell–matrix interactions that retain flavonoids under appropriate extraction conditions.

The total tannin content obtained under these extraction conditions was presented in Figure 3.

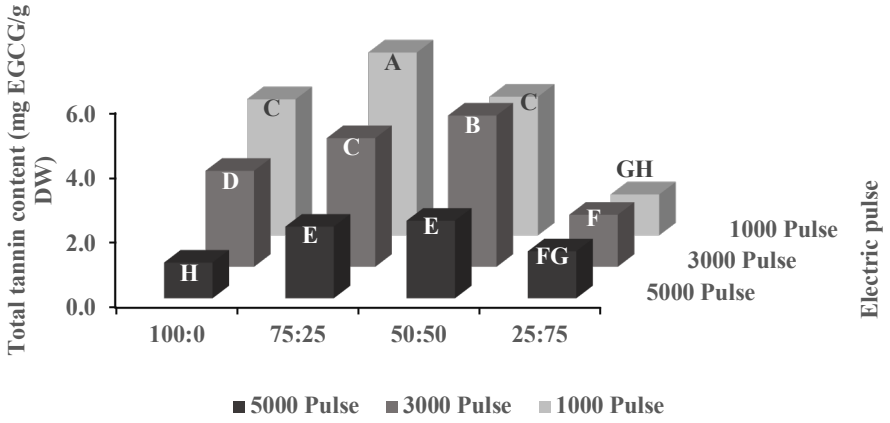


**Figure 3.** The effect of different solvent mixture ratios on total tannin content. The different letters indicate significant difference ($p < 0.05$).

The present results in figure 3 indicate that 75% ethanol with pulse number of 1,000 exhibited superior efficiency for tannin extraction compared with other solvent compositions. This observation can be explained by the chemical structure of tannins, which are polyphenolic compounds characterized by a high number of hydroxyl (–OH) groups and multiple aromatic rings. These structural features confer both polar and nonpolar properties, classifying tannins as amphipathic molecules (Koopmann, 2020). Consequently, solvent systems with intermediate polarity are more effective in dissolving tannins than highly polar or nonpolar solvents.

The water–ethanol solvent system at a concentration of 75% ethanol provides an optimal polarity for tannin solubilization. The aqueous component promotes hydrogen bond formation with hydroxyl groups of tannins, while the ethyl moiety of ethanol enhances the solubility of the aromatic structures. This synergistic effect improves the dissolution and diffusion of tannins from plant tissues,

In addition, the presence of water in the solvent system facilitates swelling of plant cell walls and disrupts interactions between tannins and macromolecules such as proteins and polysaccharides within the cell wall matrix. This structural modification enhances solvent penetration and promotes the release of tannins into the extraction medium (Mungwari et al, 2024). Previous studies have consistently reported that ethanol concentrations in the range of 50–80% are optimal for tannin extraction from various plant matrices, yielding higher extraction efficiency and antioxidant activity (Zhao et al., 2011; Jitrangsri et al., 2020).

The reducing sugar content obtained under these extraction conditions was presented in Figure 4.

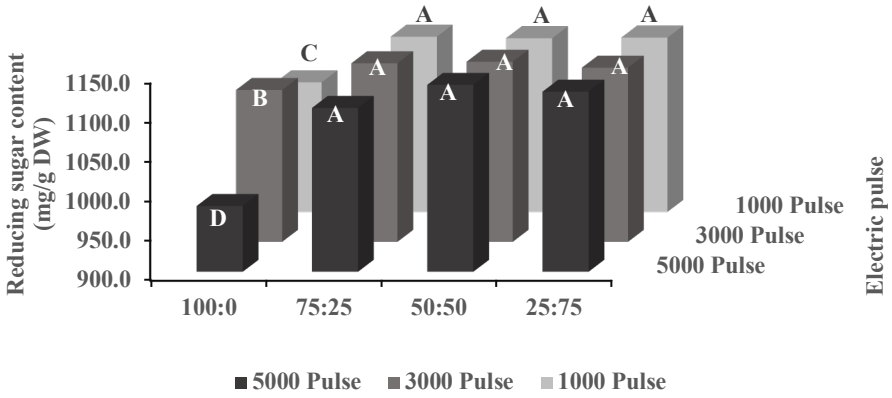


**Figure 4.** The effect of different solvent mixture ratios on reducing sugar content. The different letters indicate significant difference ($p < 0.05$).

As shown in figure 4, The enhanced extraction of reducing sugars observed in water–ethanol solvent systems may be attributed to the combined effects of solvent polarity and pulsed electric field (PEF) treatment. Reducing sugars are highly polar compounds due to the presence of multiple hydroxyl (–OH) groups, which favor solubility in polar solvents such as water. However, the incorporation of ethanol at moderate proportions can modify the overall polarity of the extraction medium, improving solvent penetration into plant tissues and facilitating mass transfer. In addition, PEF treatment induces electroporation of cell membranes, increasing membrane permeability and promoting the release of intracellular constituents as indicated by Pappas et al. (2022) and Athanasiadis et al. (2022). The synergistic effect of appropriate solvent polarity and repeated electric pulses likely enhances cell wall disruption and diffusion pathways, thereby improving the recovery of reducing sugars into the extraction medium.

The saponin content obtained under these extraction conditions was presented in Figure 5.

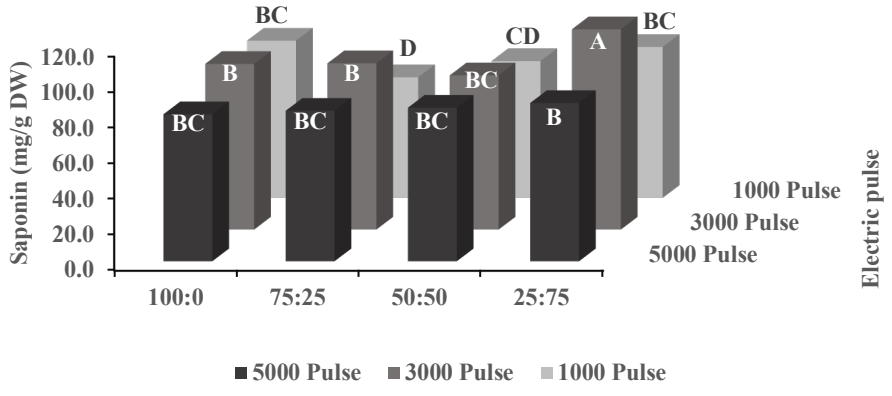


**Figure 5.** The effect of different solvent mixture ratios on saponin content. The different letters indicate significant difference ($p < 0.05$).

As shown in figure 5, the enhanced extraction of tannin observed high yield in all water–ethanol solvent systems may be attributed to the combined effects of solvent polarity and pulsed electric field (PEF) treatment. The best condition for saponin extraction appealed in 75% ethanol with pulse number of 3,000. Saponins are polar compounds that are soluble in polar solvents such as water and alcohol. The addition of an appropriate amount of water (resulting in a 50-75% concentration) increases the overall polarity of the solvent mixture compared to pure ethanol, which enhances the extraction rate by improving

compatibility with the saponin compounds as reported by Akl et al. (2022).

The saponin content obtained under these extraction conditions was presented in Figure 5.

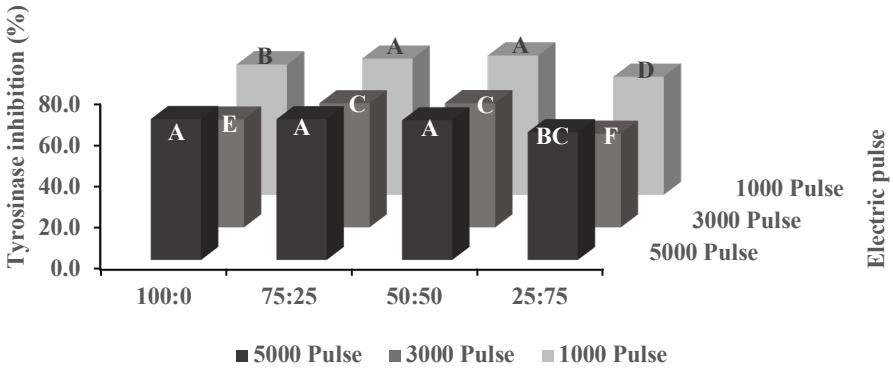


**Figure 6.** The effect of different solvent mixture ratios on tyrosinase inhibition. The different letters indicate significant difference ($p < 0.05$).

As shown in Figure 6, water and water–ethanol solvent mixtures at ratios of 75:25 and 50:50 (v/v) produced the highest tyrosinase inhibitory activity when PEF pulse numbers of 1,000 or 5,000 were applied, whereas higher ethanol concentrations did not result in comparable inhibition. This observation suggests that the efficacy of tyrosinase inhibition is influenced not only by extraction yield but also by the selective recovery of specific bioactive constituents. Cocoa pod husk (CPH) has been reported to contain a diverse combination of tyrosinase inhibitors, and synergistic interactions among these compounds may contribute to the observed bioactivity (Priani et al., 2019). Furthermore, Karim et al. (2014) demonstrated that phenolic compounds such as ferulic acid, kaempferol, procyanidins, and resveratrol exhibit significant anti-tyrosinase activity while also contributing to antioxidant properties. The enhanced inhibitory activity observed under moderate ethanol concentrations and appropriate PEF pulse numbers may therefore be attributed to the preferential extraction of these compounds. However, the present findings are not fully consistent with the report by Agudelo et al. (2021), who observed a strong correlation between total phenolic content (TPC) and tyrosinase inhibitory activity, suggesting that qualitative composition rather than total phenolic abundance may play a more critical role in tyrosinase inhibition in this system.

The antioxidant properties of CPH extracted in various conditions was also conducted and the results were presented in figure 7 and 8.

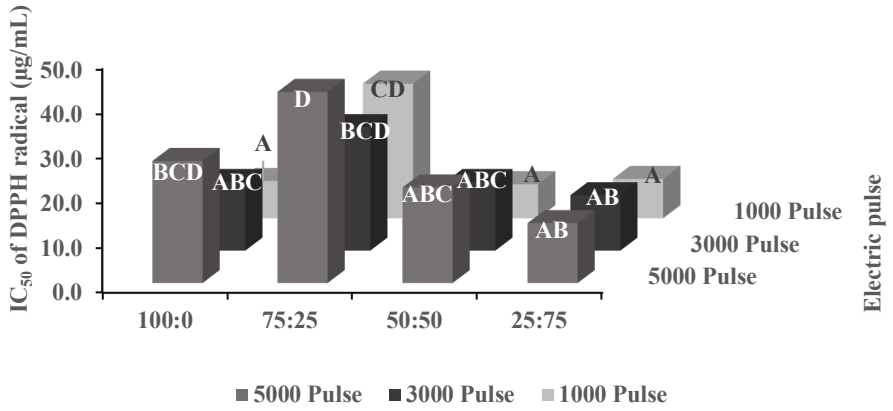


**Figure 7.** The effect of different solvent mixture ratios on antioxidant activities measured by the DPPH assay. The different letters indicate significant difference ($p < 0.05$). The lower $IC_{50}$ value in a DPPH assay indicates a stronger antioxidant

The DPPH radical-scavenging assay is widely recognized as a valid, accurate, simple, and cost-effective method for evaluating the antioxidant capacity of water-soluble compounds. As shown in Figure 7, the highest antioxidant activity was observed in extracts obtained using water and water–ethanol solvent mixtures at ratios of 50:50 and 25:75 (v/v) when a PEF pulse number of 1,000 was applied. This enhanced antioxidant activity may be attributed to the synergistic action of multiple bioactive compounds such as catechin, epicatechin, procyanidin B2, and clovamide (Gomez et al., 2025) with differing polarity characteristics, whereby highly polar antioxidants are efficiently extracted in aqueous systems, while moderately polar compounds require hydroethanolic conditions for optimal solubilization.

Water–ethanol mixtures containing 50–75% ethanol provides intermediate polarity, which is particularly suitable for dissolving a wide range of antioxidant compounds, including phenolic acids (e.g., ferulic acid, gallic acid and resveratrol), polyphenols (e.g., protocatechuic acid, p-hydroxybenzoic acid), flavonoids (e.g., quercetin and kaempferol), as well as proanthocyanidins and tannins (Anatachodwanit et al., 2025; Belwal et al., 2020). The combined presence of these compounds likely contributes to the superior radical-scavenging capacity observed under these extraction conditions. To further corroborate the antioxidant potential of the extracts, ferric reducing antioxidant power (FRAP) assays were performed, and the results are presented in Figure 8.

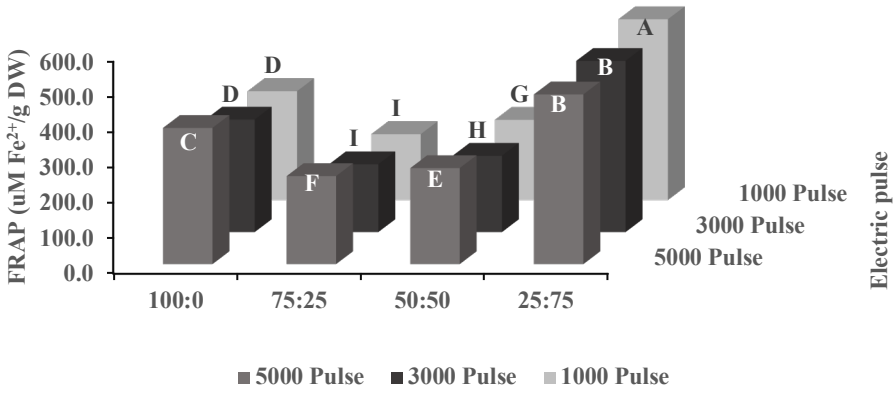


**Figure 8.** The effect of different solvent mixture ratios on antioxidant activities measured by the FRAP assay. The different letters indicate significant difference ($p < 0.05$). The higher value indicates a stronger antioxidant

The ferric reducing antioxidant power (FRAP) assay is a widely used colorimetric method for assessing total antioxidant capacity based on the ability of antioxidants to donate electrons and reduce ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$) under acidic conditions, reflects the reducing power of the sample and is commonly expressed as $Fe^{2+}$ equivalents. As shown in Figure 8, the highest FRAP values were observed in extracts obtained using a water–ethanol solvent mixture at a ratio of 25:75 (v/v) when a PEF pulse number of 1,000 was applied.

The enhanced reducing capacity detected under these extraction conditions may be attributed to the synergistic contribution of multiple bioactive compounds with different polarity characteristics. Highly polar antioxidants are preferentially extracted in aqueous environments, whereas moderately polar compounds are more effectively solubilized in hydroethanolic systems with intermediate polarity. Consequently, the 25:75 (v/v) water–ethanol mixture likely promoted the co-extraction of a broader spectrum of redox-active compounds, resulting in elevated FRAP values. These findings are consistent with the antioxidant trends observed in the DPPH assay in the present study and are in agreement with the report by Vu et al. (2025), thereby reinforcing the reliability of the antioxidant assessment and the effectiveness of the selected extraction conditions.

## CONCLUSIONS

This study demonstrated that pulsed electric field (PEF)–assisted extraction combined with green water–ethanol solvents is an effective approach for recovering bioactive compounds from cocoa pod husk (CPH). The extraction efficiency of phenolics,

flavonoids, tannins, reducing sugars, and antioxidant activity was strongly influenced by solvent polarity and PEF pulse numbers. Water–ethanol mixtures with intermediate polarity, particularly 50–75% ethanol, consistently yielded higher levels of bioactive compounds and antioxidant capacity. The application of PEF enhanced cell membrane permeabilization, facilitating mass transfer and improving the release of intracellular constituents. Antioxidant activity evaluated by DPPH and FRAP assays showed consistent trends, confirming the reliability of the extraction outcomes. Moderate PEF pulse numbers (1,000–3,000 pulses) were sufficient to achieve optimal extraction without excessive degradation of sensitive compounds. Tyrosinase inhibitory activity was maximized under hydroethanolic conditions, indicating the presence of multiple bioactive inhibitors acting synergistically. The lack of direct correlation between total phenolic content and tyrosinase inhibition suggests that specific compound composition plays a critical role in bioactivity. Overall, the findings highlight the importance of balancing solvent polarity and PEF parameters to maximize extraction efficiency. This PEF-assisted green extraction strategy offers a sustainable and scalable approach for valorizing cocoa processing by-products. The extracted compounds exhibit strong potential for applications in food, cosmetic, and cosmeceutical industries. Future studies should focus on compound identification and scale-up optimization to further support industrial implementation.

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