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Comparative Study on Ultrasonic and Pulsed Electric Field Extraction of Phrayawan (Curcuma Phrayawan Boonma & sansouk) Rhizomes and Evaluation of Bioactive

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Abstract

This study investigated the effects of Ultrasonic-Assisted Extraction (UAE), Pulsed Electric Field (PEF), and their sequential combinations on the extraction efficiency, phytochemical content, and antioxidant activities of Curcuma phrayawan Boonma & Saensouk rhizomes. Rhizomes aged 6-8 months were dried, powdered, and extracted using deionized water, 50% ethanol, and 99.7% ethanol under four extraction conditions: UAE, PEF, UAE + PEF, and PEF + UAE. All extractions were performed in triplicate (n=3), and results were expressed as mean ± standard deviation. Extraction yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH, ABTS, and FRAP assays) were evaluated. The UAE + PEF technique using deionized water produced the highest extraction yield (10.32 ± 0.79%), whereas PEF + UAE with 99.7% ethanol achieved the highest TPC (494.69 ± 0.20 mg GAE/g) and TFC (194.51 ± 0.82 mg QE/g). Antioxidant activities correlated positively with TPC and TFC, with PEF + UAE extracts exhibiting the strongest radical scavenging and reducing power (DPPH IC50 = 712.53 ± 6.91 µg/mL, ABTS IC50 = 651.73 ± 5.22 µg/mL, FRAP IC50 = 373.67 ± 3.79 µg/mL). The enhanced performance of the combined UAE and PEF techniques can be attributed to the synergistic effects of acoustic cavitation and electroporation, which improve solvent penetration, cell wall disruption, and mass transfer. These findings demonstrate that integrating UAE and PEF provides a green, efficient, and scalable extraction strategy for producing bioactive-rich C. phrayawan extracts with potential applications in functional foods, nutraceuticals, and cosmetics.

Keywords

Curcuma Phrayawan Boonma & Saensouk, Ultrasonic-Assisted Extraction, Pulsed Electric Field, Antioxidant Activity

Introduction

Herbal plants have gained increasing global attention due to their important roles in health promotion and disease prevention. Their rich species diversity, abundance of bioactive compounds, and long-standing traditional knowledge contribute to their recognized therapeutic potential and relative safety, while growing consumer preference for natural-based products continues to expand the global herbal market. Thailand is recognized as one of the most biodiverse countries in terms of medicinal plant resources, with a wide variety of herbal species traditionally used in medicine. To support this sector, the Thai government has implemented national policies promoting herbal production for domestic use and international markets, including the National Master Plan for Thai Herbal Development (2017–2021), which aims to conserve traditional knowledge, improve the quality of herbal raw materials, and encourage the efficient utilization of herbal resources. Despite these efforts, the Thai herbal industry still relies largely on primary processing and the production of

raw or minimally processed materials, limiting the development of high-value and innovative herbal products [1].

The Thai herbal market has continued to expand across several product categories, particularly functional beverages, herbal remedies for respiratory symptoms such as coughs and colds, and herbal dietary supplements. Between 2017 and 2019, these product groups experienced an average growth rate of approximately 10.3%, which exceeded the growth rates of major herbal markets in Asia, including China, Japan, and South Korea. This rapid expansion highlights Thailand's strong potential to become a competitive player in the regional herbal economy.

In addition to market growth, botanical exploration and taxonomic research in Thailand have contributed to the discovery of several new medicinal plant species. Researchers from Mahasarakham University, with support from the National Science and Technology Development Agency (NSTDA), reported several new species within the Zingiberaceae family, particularly in the genera Curcuma and Kaempferia. These discoveries reflect the rich biodiversity of medicinal plants in Thailand and highlight the potential for identifying new plant resources with pharmaceutical and nutraceutical value [2].

Among these species, Curcuma phrayawan Boonma & Saensouk, commonly known as "Phaya Wan," has attracted increasing attention due to its potential medicinal properties. The plant exhibits morphological characteristics similar to turmeric, including a yellow rhizome, bitter taste, white flowers, a red pseudostem, and a mild aromatic scent. In Thai traditional medicine, C. phrayawan is believed to possess various therapeutic properties, including detoxification effects, cardiovascular support, and relief of symptoms associated with the eyes, throat, nose, digestive system, and skin [3].

Despite its ethnomedicinal importance, scientific studies on C. phrayawan remain limited. In particular, there is still a lack of information regarding efficient extraction techniques, phytochemical composition, and biological activities such as antioxidant capacity. Therefore, further investigation is necessary to explore its potential as a valuable source of bioactive compounds.

The extraction process plays a crucial role in determining the yield and quality of bioactive compounds obtained from plant materials. Conventional extraction techniques often require long processing times and large volumes of solvents, which may reduce extraction efficiency and increase processing costs. In contrast, modern extraction technologies have been developed to improve extraction efficiency while preserving thermolabile compounds.

Ultrasonic-assisted extraction (UAE) is an emerging technique that utilizes ultrasonic waves to generate acoustic cavitation. This phenomenon disrupts plant cell walls and enhances the release of intracellular compounds into the extraction solvent [4]. Another promising technique is pulsed electric field (PEF) extraction, a non-thermal technology that applies short pulses of high voltage to plant tissues. This process increases cell membrane permeability through electroporation, thereby facilitating the release of bioactive compounds [5].

The integration of UAE and PEF technologies may provide synergistic effects due to their different mechanisms of action. While UAE enhances mass transfer through cavitation-induced cell disruption, PEF promotes the permeabilization of cell membranes. The combination or comparison of these advanced extraction approaches may therefore improve the efficiency of phytochemical recovery from medicinal plants [6].

Therefore, this study aimed to compare ultrasonic-assisted extraction (UAE) and pulsed electric field (PEF) extraction for the recovery of bioactive compounds from C. phrayawan rhizomes and to evaluate the antioxidant activities of the resulting extracts. The findings of this research are expected to provide useful insights into efficient extraction techniques for this medicinal plant and contribute to the development of value-added herbal products in the Thai herbal industry.

Materials and Methods

Sample Preparation

Rhizomes of C. phrayawan aged 6-8 months were collected from Maha Sarakham Province, Thailand. The rhizomes were thoroughly washed with distilled water to remove soil and other impurities and then sliced into small pieces (approximately 0.2-0.5 cm in thickness). The sliced samples were dried in a hot air oven at 50 °C until a constant weight was obtained, corresponding to a final moisture content of approximately 10%.

The dried rhizomes were subsequently ground into fine powder using a laboratory grinder and passed through a 40-mesh sieve to obtain a uniform particle size. The powdered samples were packed in aluminum foil bags, sealed to prevent moisture absorption, and stored at a temperature below 4 °C until further analysis.

Materials

Gallic acid, quercetin, aluminum chloride, sodium carbonate, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent was obtained from Merck (Germany). Ethanol and other analytical-grade solvents were purchased from RCI Labscan (Thailand). Deionized water was used throughout the experiments.

Extraction Process of C. Phrayawan

The dried rhizome powder of C. phrayawan was extracted using three different solvents, namely deionized water (DI), 50% ethanol, and 99.7% ethanol (v/v), at a solid-to-solvent ratio of 1:10 (w/v). The mixtures were subjected to different extraction techniques to evaluate the efficiency of bioactive compound recovery. Ultrasonic-assisted extraction (UAE) was performed using an ultrasonic bath (UC-360D, China) operating at a power of 250 W for 25 min while maintaining the extraction temperature below 40 °C. Pulsed electric field (PEF) treatment was conducted using a custom-built pulsed electric field system developed at Payap University, Chiang Mai, Thailand, where the extraction mixture was treated with an electric field strength of 2 kV/cm with 49 pulses. To compare the efficiency of different extraction approaches, four extraction methods were investigated: UAE, PEF, UAE followed by PEF (UAE + PEF), and PEF followed by UAE (PEF + UAE). Each extraction method was carried out using the three solvents under identical experimental conditions.

After extraction, the mixtures were filtered through Whatman No. 1 filter paper, and the resulting extracts were collected and stored at −20°C until further analysis. All experiments were conducted in triplicate [4, 7].

Determination of Total Phenolic Content

The total phenolic content (TPC) of C. phrayawan extracts was determined using the Folin–Ciocalteu colorimetric method with slight modifications. Gallic acid was used as the standard and prepared in 95% ethanol. A calibration curve was constructed using gallic acid concentrations ranging from 0–200 µg/mL. The extract samples were also diluted with 95% ethanol prior to analysis. Briefly, 0.3 mL of each standard or sample solution was mixed with 1.5 mL of Folin–Ciocalteu reagent and 1.2 mL of 7.5% Na_2CO_3 solution. The mixture was incubated at room temperature in the dark for 30 min. The absorbance was measured at 725 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan). The TPC was calculated from the gallic acid calibration curve and expressed as mg GAE/g extract. All measurements were performed in triplicate [8].

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method [8]. Quercetin was used as the standard to construct a calibration curve with concentrations ranging from 0-100 µg/mL. Briefly, 100 µL of the sample solution (1000 µg/mL) was added into a 96-well microplate, followed by the addition of 100 µL of 2% $AlCl_3$ solution. The mixture was gently mixed and allowed to stand at room temperature for 15 min. The absorbance was measured at 405 nm using a microplate reader (Spark, Tecan, Switzerland). The TFC was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). All measurements were performed in triplicate.

Determining Antioxidant Activity

The antioxidant activity of the extracts was evaluated using three standard assays: DPPH radical scavenging, ABTS radical cation decolorization, and ferric reducing antioxidant power (FRAP) assays. All measurements were performed using a microplate reader.

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity was determined with slight modification from the method of Tanzadehpanah et al. [9]. Briefly, 20 µL of each sample solution was mixed with 180 µL of 0.2 mM DPPH solution prepared in ethanol. The mixture was vortexed for 10 s and incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a microplate reader.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The antioxidant activity of the extracts was expressed as IC50 values (µg/mL), defined as the concentration required to inhibit 50% of DPPH radicals. The IC_{50} values were calculated from the plotted graph of DPPH inhibition (%) against sample concentration. All measurements were performed in triplicate.

ABTS Radical Cation Decolorization Assay

The ABTS assay was carried out according to the modified method of Re et al. [10]. The ABTS radical cation ($ABTS^{\bullet+}$) was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate in a 1:1 ratio and allowing the mixture to stand in the dark for 12-16 hours at room temperature. A 20 µL aliquot of the sample solution was added to 180 µL of the $ABTS^{\bullet+}$ working solution and mixed by vortexing. The reaction was incubated at 37°C for 6 minutes in the dark, and absorbance was measured at 734 nm. The percentage inhibition was calculated using the same formula as above.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed following the method of Benzie and Strain [11] with slight modification. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio (v/v/v). A 10 µL aliquot of the sample was added to 190 µL of FRAP reagent, vortexed briefly, and incubated at 37°C for 30 minutes in the dark. The reducing power was expressed as percentage of antioxidant activity (%). The IC50 value, defined as the concentration required to reach 50% reducing activity, was calculated from the dose-response curve.

$$\%\text{Reduction power} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of sample}} \times 100$$

Determining Antioxidant Activity

All data are expressed as mean ± SD (n=3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). IC_{50} values were calculated by nonlinear regression using GraphPad Prism (GraphPad Software Inc., USA). Statistical significance was set at $p < 0.05$.

Results & Discussion

Extraction Yield

The extraction yield of C. phrayawan rhizome extracts obtained using four extraction techniques ultrasonic-assisted extraction (UAE), pulsed electric field (PEF), UAE followed by PEF (UAE + PEF), and PEF followed by UAE (PEF + UAE) with three different solvents (99.7% ethanol, 50% ethanol, and deionized water) was evaluated. All extractions were performed in triplicate, and the results are summarized in Table 1. Among all conditions, the highest extraction yield was obtained using the UAE + PEF technique with deionized water as the solvent, yielding 10.32 ± 0.79%, followed by PEF + UAE using 99.7% ethanol (9.93 ± 0.47%). Other techniques produced slightly lower yields, indicating that the sequential combination of ultrasonic and electric field treatments significantly enhances extraction efficiency compared to applying either method alone.

The superior extraction yield observed in UAE + PEF with deionized water can be attributed to the synergistic effect of both techniques. Ultrasonic-assisted extraction (UAE) generates cavitation, which disrupts plant cell walls, while pulsed electric field (PEF) induces electroporation, increasing membrane permeability and facilitating solvent penetration. This dual action

enhances mass transfer of intracellular compounds into the solvent, resulting in higher extraction efficiency. Similar synergistic effects of combining UAE and PEF have been reported by Sai Krishna et al. (2023) and Hoang Le-Tan et al. (2022), who found that sequential treatment improved extraction yield and reduced extraction time [4, 7].

Additionally, deionized water, being a highly polar solvent, may preferentially extract hydrophilic compounds from C. phrayawan, contributing to higher yields under certain conditions compared to ethanol. These results suggest that both solvent polarity and extraction technique play critical roles in maximizing recovery of bioactive compounds.

Table 1: Extraction yield of C. phrayawan rhizome extracts under different extraction techniques and solvents.

Extraction Technique	Extraction Yield (%)		
	99.7% ethanol	50% ethanol	Deionized Water
UAE	9.07±1.10b	6.77±0.26b	7.88±0.89b
PEF	9.05±0.87b	5.87±0.08c	9.29±0.71ab
UAE + PEF	9.73±0.97ab	7.17±0.62b	10.32±0.79a
PEF+ UAE	9.93±0.47a	8.22±0.49a	7.59±0.92b

Different superscript letters within each column indicate significant differences ($p < 0.05$).

Total Phenolic Content (TPC)

The total phenolic content (TPC) of C. phrayawan extracts obtained using four extraction techniques ultrasonic-assisted extraction (UAE), pulsed electric field (PEF), UAE followed by PEF (UAE + PEF), and PEF followed by UAE (PEF + UAE) with three solvents (50% ethanol, 99.7% ethanol, and deionized water) was evaluated. All extractions were performed in triplicate (n=3), and results are expressed as mean ± SD (Table 2).

Among all conditions, PEF + UAE using 99.7% ethanol yielded the highest TPC (494.69 ± 0.20 mg GAE/g sample), significantly higher than the other extraction methods ($p < 0.05$). PEF and UAE alone gave comparable yields (476.14 ± 0.07 and 475.67 ± 0.19 mg GAE/g, respectively), while UAE + PEF produced the lowest TPC (457.82 ± 0.17 mg GAE/g). Moderate TPC values were observed with 50% ethanol, whereas deionized water resulted in markedly lower yields (<100 mg GAE/g), indicating that high-concentration ethanol is a more effective solvent for phenolic extraction.

The superior performance of PEF + UAE can be attributed to the synergistic effect of both techniques: PEF induces electroporation, enhancing cell membrane permeability, while UAE generates cavitation that disrupts cell walls, facilitating mass transfer of intracellular phenolics. These findings are consistent with Pitivitayakul et al. (2020) [8], who reported that ethanolic extracts of Curcuma zedoaria and Curcuma comosa exhibited the highest TPC and strongest antioxidant activities. Overall, sequential PEF + UAE using 99.7% ethanol was the most effective method for extracting phenolic compounds from C. phrayawan.

Table 2: Total phenolic contents (TPC) of C. phrayawan rhizome extracts under different extraction techniques and solvents.

Extraction Technique	Total Phenolic Compounds (mg GAE/g sample)		
	99.7% ethanol	50% ethanol	Deionized Water
UAE	475.67±0.19b	148.29±0.12d	81.86±0.031a
PEF	476.14±0.07b	386.90±0.17a	41.43±0.03c
UAE + PEF	457.82±0.17c	271.13±0.13b	45.23±0.05b
PEF+ UAE	494.69±0.20a	226.49±0.14c	35.13±0.01d

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of C. phrayawan rhizome extracts obtained using four extraction techniques ultrasonic-assisted extraction (UAE), pulsed electric field (PEF), UAE followed by PEF (UAE + PEF), and PEF followed by UAE (PEF + UAE) with three solvents (50% ethanol, 99.7% ethanol, and deionized water) was evaluated. All extractions were performed in triplicate (n=3), and results are expressed as mean ± SD.

As shown in Table 3, the TFC followed a trend similar to total phenolic content (TPC). The PEF + UAE technique using 99.7% ethanol produced the highest flavonoid yield (194.51 ± 0.82 mg QE/g sample), followed by UAE (190.14 ± 0.04 mg QE/g) and PEF (184.07 ± 0.11 mg QE/g). UAE + PEF showed the lowest value (179.79 ± 0.72 mg QE/g) within this solvent group. Using 50% ethanol, PEF yielded the highest TFC (168.75 ± 0.78 mg QE/g), while all extractions with deionized water resulted in markedly lower yields, with UAE achieving the highest among them (15.02 ± 0.58 mg QE/g). These results indicate that high-concentration ethanol (99.7%) is a more effective solvent for flavonoid extraction from C. phrayawan rhizomes.

The superior performance of PEF + UAE can be explained by the synergistic effect of the two techniques: PEF induces electroporation, increasing cell membrane permeability, and UAE generates cavitation that disrupts cell walls, enhancing flavonoid release and solvent penetration. Lower yields with deionized water are attributed to the poor solubility of flavonoids in highly polar aqueous media. These findings are consistent with previous reports by Pitivitayakul et al. (2020) [8] demonstrating that

sequential PEF and UAE treatments improve flavonoid recovery in plant matrices. Overall, PEF + UAE using 99.7% ethanol was the most efficient method for flavonoid extraction from C. phrayawan rhizomes.

Table 3: Total flavonoid contents (TFC) of C. phrayawan rhizome extracts under different extraction techniques and solvents.

Extraction Technique	Total Flavonoid Contents (mg QE/g sample)		
	99.7% ethanol	50% ethanol	Deionized Water
UAE	190.14±0.04a	58.46±0.53c	15.02±0.58a
PEF	184.07±0.11b	168.75±0.78a	6.68±0.05b
UAE + PEF	179.79±0.72c	95.31±0.51b	8.29±0.05b
PEF+ UAE	194.51±0.82a	83.52±0.59b	6.12±0.02b

Different superscript letters within each column indicate significant differences ($p < 0.05$).

Selection of the Optimum Extraction Technique for Antioxidant Activity Evaluation

The results revealed that both extraction technique and solvent type significantly influenced the extraction efficiency and bioactive compound content of C. phrayawan extracts. Although the UAE + PEF method using deionized water provided the highest extraction yield (10.32 ± 0.79%), it exhibited the lowest total phenolic and flavonoid contents. In contrast, the PEF + UAE extraction using 99.7% ethanol achieved the highest total phenolic content (494.69 ± 0.20 mg GAE/g) and total flavonoid content (194.51 ± 0.82 mg QE/g), indicating superior efficiency in extracting polyphenolic and flavonoid content, which are key contributors to antioxidant activity [13]. Therefore, the PEF + UAE technique with 99.7% ethanol is considered the most appropriate method for evaluating the antioxidant activity of C. phrayawan extracts, as it yields the highest concentrations of antioxidant-related compounds, consistent with the findings of Pitivitayakul et al. (2020) [13], who reported that ethanolic extracts of C. zedoaria and C. comosa with higher phenolic contents exhibited stronger antioxidant capacities than those extracted with other solvents.

Antioxidant Activity

The antioxidant activities of C. phrayawan rhizome extracts obtained using the PEF + UAE (P+U) technique were evaluated using DPPH, ABTS, and FRAP assays. All experiments were performed in triplicate (n=3), and the results are expressed as mean ± standard deviation (SD) (Table 4).

The IC50 values of the P+U extract were significantly higher than those of Trolox, a standard antioxidant compound, in all assays ($p < 0.05$), indicating lower radical scavenging and reducing capacities compared with the positive control. Specifically, the IC50 value of the extract in the DPPH assay was 712.53 ± 6.91 µg/mL, whereas Trolox exhibited an IC50 of 100.25 ± 3.78 µg/mL. In the ABTS assay, the extract showed an IC50 value of 651.73 ± 5.22 µg/mL compared with 59.31 ± 1.22 µg/mL for Trolox. Similarly, in the FRAP assay, the extract exhibited an IC50 value of 373.67 ± 3.79 µg/mL, while Trolox demonstrated a substantially lower IC50 of 25.16 ± 0.64 µg/mL. These findings indicate statistically significant differences in antioxidant activity between the C. phrayawan extract and Trolox across all assays, confirming that the extract possesses comparatively lower antioxidant capacity.

The relatively high IC50 values observed for the P+U extract may be attributed to the concentration and composition of bioactive compounds present in the rhizome, which are generally less potent on a mass basis than pure antioxidant standards such as Trolox. Nevertheless, the extract still exhibited measurable radical scavenging and reducing activities, suggesting its potential as a natural antioxidant source when applied at appropriate concentrations.

The antioxidant activity detected in the C. phrayawan extract may be associated with the presence of bioactive phytochemicals, particularly phenolic compounds and curcuminoids, which are widely reported in Curcuma species and contribute to their antioxidant properties [13]. Previous studies have also demonstrated that rhizome extracts of Curcuma species obtained using green extraction techniques exhibit detectable antioxidant activities due to these phytochemical constituents [14]. Although plant extracts generally show lower antioxidant activity than pure standards such as Trolox, they can still provide biologically relevant radical scavenging and reducing capacities [15].

Table 4: Antioxidant activities (IC50) of C. phrayawan rhizome extracts and Trolox.

Sample	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP IC50 (µg/mL)
Trolox	100.25 ± 3.78b	59.31 ± 1.22b	25.16 ± 0.64b
PEF+UAE	712.53 ± 6.91a	651.73 ± 5.22a	373.67 ± 3.79a

Values are presented as mean ± SD (n = 3). Different superscript letters within each column indicate significant differences ($p < 0.05$).

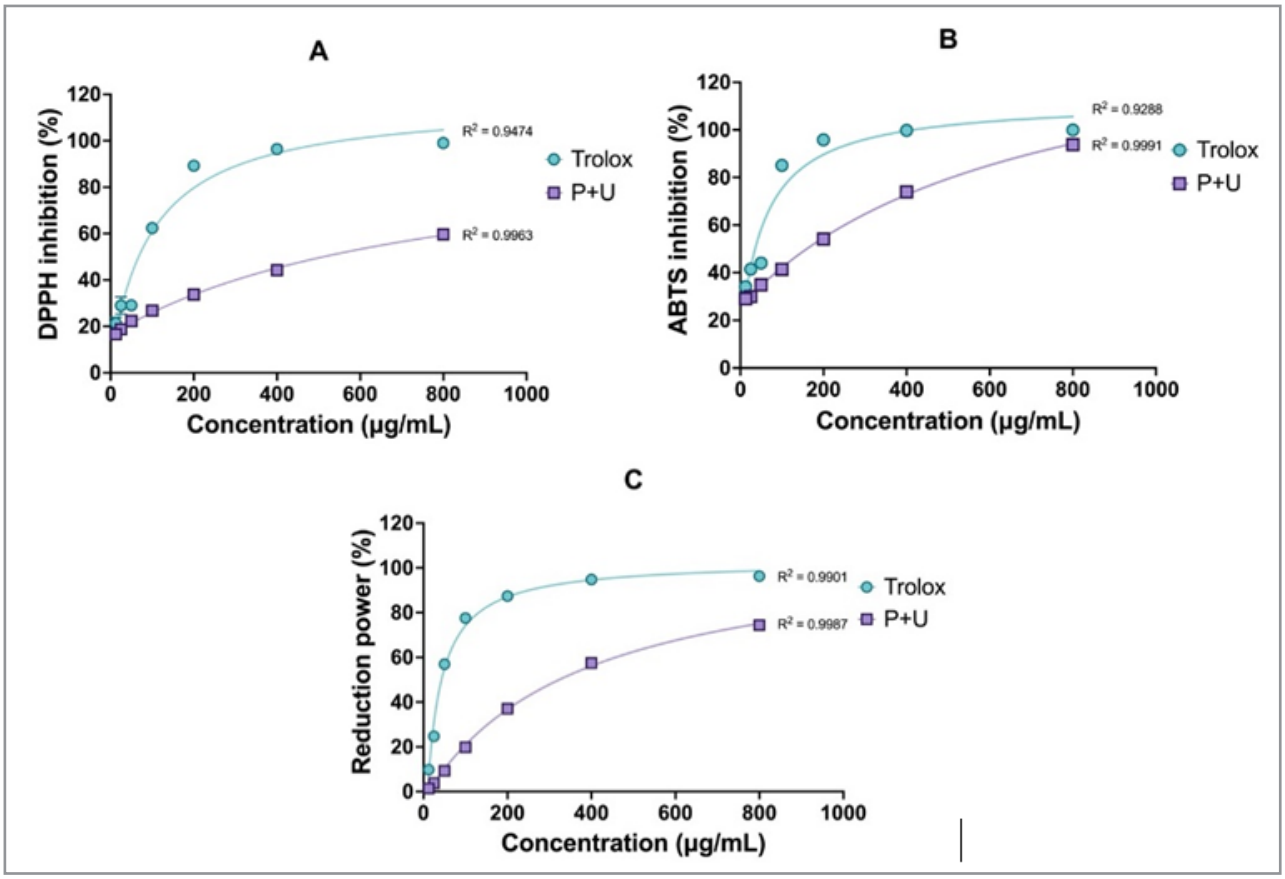

Figure 1: Nonlinear regression curves used for the determination of IC_{50} values of Trolox and C. phrayawan extract obtained by pulsed electric field followed by ultrasonic-assisted extraction (P+U). Antioxidant activities were evaluated using (A) DPPH, (B) ABTS, and (C) FRAP assays.

Conclusion

This study demonstrates that both extraction technique and solvent type play critical roles in determining the efficiency of bioactive compound recovery from C. phrayawan rhizomes. Among the investigated approaches, the sequential application of pulsed electric field followed by ultrasonic-assisted extraction (PEF + UAE) using 99.7% ethanol provided the most effective recovery of phenolic and flavonoid compounds. The enhanced extraction efficiency of this method is likely attributed to the synergistic effects of electroporation and acoustic cavitation, which facilitate cell wall disruption and improve solvent penetration, thereby promoting the release of intracellular phytochemicals.

Although the antioxidant activity of the extract was lower than that of the Trolox standard, the extract still exhibited appreciable radical scavenging and reducing capacities in DPPH, ABTS, and FRAP assays, indicating its potential as a natural antioxidant source. Overall, the PEF + UAE technique using ethanol represents an efficient strategy for enhancing the recovery of antioxidant-related phytochemicals from C. phrayawan rhizomes. These findings provide valuable insights for the development of antioxidant-rich herbal extracts with potential applications in nutraceutical and cosmetic industries. Further studies focusing on compound characterization and process optimization may facilitate future industrial applications.

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