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Enhancement of Phenolic Extraction and Antioxidant Activity from Field Corn Cobs Using Ultrasound-Assisted Extraction for Sustainable Valorization

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ABSTRACT

Field corn cobs, a major agricultural byproduct, are rich in phenolic compounds with antioxidant potential. The aim of this study was to optimize phenolic extraction from field corn cobs using ethanol and ultrasound-assisted ethanol extraction (UAE). Compared to conventional extraction, UAE improved efficiency while reducing time. The optimal condition for phenolic extraction was using UAE with 50% ethanol, 37 kHz frequency, 50°C, and 60 minutes. The field corn cob extract showed the highest total phenolic content at 1125.38 µg GAE/g CC, and the inhibitory concentration (IC_{50}) of DPPH was 2.48 mg/mL. Even though the IC_{50} value of field corn cob extract is higher than that of ascorbic acid (29.16 µg/mL). This research highlights the sustainable use of corn cobs as a natural antioxidant source for industrial applications.

1. Introduction

Humans and animals use corn (*Zea mays* L.) as a cereal grain [1]. In Thailand, field corn is an essential raw ingredient for the feed industry, holding significant economic value, with an annual export worth around 73 million USD [2]. Currently, a significant challenge in field corn cultivation is managing the residues after harvesting. After milling the corn seeds, field corn cobs remain as agricultural waste. Most farmers dispose of these residues by burning, which contributes to toxic pollution, including fine particulate matter (PM2.5), especially in northern Thailand [3-4]. To lessen the damage to the environment, many studies have investigated using field corn cob leftovers for different things, like making ethanol and xylitol [5], binder [6], fiber for feed [7], and biomass [8-9]. However, limited research exists on phenolic extraction from field corn cobs in Thailand. This study investigates the extraction of phenolic compounds from agricultural field corn cob residues.

Prior investigations have indicated that corn cobs serve as a superior source of antioxidants in comparison to other agricultural by-products, including peanut hulls,

wheat bran, and sugarcane bagasse [10]. Additionally, they exhibited higher antioxidant activity than corn husks, grain, roots, leaves, and stems (*Zea mays* var. Pioneer W3254) [11]. In vitro, the phenolic compounds and flavonoids in corn cob extract can fight inflammation, bacteria, free radicals, and cancer [12-13]. The extract derived from corn cobs exhibits considerable promise as a natural antioxidant, which may serve as a viable substitute for synthetic antioxidants, including butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), in certain applications. Considering these bioactivities, corn cob extracts possess potential applications such as natural antioxidants in food preservation[14],dietary supplements, and pharmaceuticals [15].

When compared between conventional extraction methods for phenolic compounds, which encompass solvent extraction (employing water, methanol, ethanol, or acetone), maceration, and infusion, there exists a clear divergence from modern techniques. Modern alternative extraction methods incorporate ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE). Microwave and ultrasound extraction techniques have recently become popular for extracting phenolic compounds due to their efficiency, speed, and energy conservation advantages [16-17]. Prior research indicates that the UAE method outperforms conventional techniques in extracting phenolic compounds from oleuropein in olive leaves [18] and mandarin peel [19]. The UAE demonstrated greater efficacy than MAE in the extraction of phenolic compounds and exhibited superior antioxidant activity [20]. A binary ethanol-water solvent demonstrates greater efficacy than a single solvent for the extraction of phenolics [21]. Ethanol interferes with solute-matrix interactions, whereas water facilitates cell swelling, thereby increasing phenolic release [22]. This combination enhances molecular polarity, thereby increasing phenolic solubility and extraction efficiency [23]. Even though numerous investigations on phenolic extraction in the UAE have been accomplished, studies regarding its utilization for field corn cobs, particularly in Thailand, are a small number. Because of these reasons, the goal of this study was to optimize the extraction of phenolic compounds from field corn cobs using UAE and ethanol solvents, making them suitable for industrial applications and valuable economic purposes. Additionally, the field corn cob extract was produced on a large scale and processed via freeze-drying into a powdered form for the assessment of its antioxidant efficacy in comparison to ascorbic acid.

2. Experiments

2.1. Chemicals

Gallic acid (98%, Sigma-Aldrich, USA), ascorbic acid (98%, Ajax Finechem, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%, Sisco Research Laboratories, India), sodium carbonate (99.8%, Ajax Finechem, Germany), Folin-Ciocalteu reagent (99%, Sisco Research Laboratories, India), ethanol (99.9%, RCI Labscan, Thailand).

2.2. Preparation of field corn cobs

Field corn cobs were collected post-harvest in April 2018 from Banmo, Phichai, Uttaradit. They were washed, oven-dried at 50°C for 24 hours, and pulverized into fine powder using an electric grinder. The powder was stored in airtight containers under dry conditions for further experiments.

2.3. Conventional extraction of field corn cobs

A total of 10 g of field corn cob powder was mixed with 150 mL of 50% ethanol and incubated at 50°C in a temperature-controlled shaker at 150 rpm. The extraction time varied at 30, 60, 120, 240 and 360 minutes. The

mixture was filtered through vacuum filtration and the solvent was removed using a rotary evaporator (BUCHI/R-210, Switzerland). The extract was adjusted to 10 mL with distilled water and stored in amber bottles at 4°C for further analysis.

2.4. Ultrasound-assisted ethanol extraction (UAE) of field corn cobs

A total of 10 g of field corn cob powder was mixed with 150 mL of 50% ethanol and extracted using an ultrasonic water bath (S100H, ELMA, Germany) at 37 kHz and 50°C for 15, 30, and 60 minutes. The extract was filtered, evaporated, and adjusted to 10 mL before storage at 4°C for further analysis like previous experiments.

2.5. Preparation of field corn cob extract powder

After determining the optimal extraction conditions that resulted in the highest phenolic content and DPPH inhibition, a large-scale extraction was performed to confirm the total phenolic content and antioxidant activity of the field corn cob extract powder in comparison with standard ascorbic acid. For this, 500 g of field corn cobs were extracted using 7.5 L of 50% ethanol. The extract was then filtered, and the solvent was removed by evaporation to obtain a concentrated solution. This solution was subsequently freeze-dried using a freeze dryer (Labogene Coolsafe, Denmark) at -20°C and -70°C. Finally, the obtained extract powder was analyzed to determine its total phenolic content (expressed as μg GAE/g CC) and antioxidant activity, measured as the IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals.

2.6. Determination of Total Phenolic Content (TPC)

TPC was quantified using the Folin-Ciocalteu method with gallic acid as the standard, as described by Mokrani et al. [24]. 0.5 mL of Folin-Ciocalteu reagent was added in 0.5 mL of the corn cob extract solution in a test tube and thoroughly mixed. Subsequently, 4 mL of a 15% sodium carbonate solution was added and thoroughly mixed. After a 30-minute reaction of the mixture solution, the absorbance was measured at 760 nm using a spectrophotometer (Genesys 20, Thermo Scientific, USA). The total phenolic content was determined using a gallic acid standard curve at concentrations of 20, 40, 60, 80, and 100 μg/mL ($y = 13.944x$, $R^2 = 0.9994$). The phenolic content of field corn cob extract was defined as micrograms of gallic acid equivalents per gram of dry corn cob (μg GAE/g CC).

2.7. Analysis of Antioxidant Activity

The antioxidant activity was determined using the DPPH radical scavenging assay, based on Mokrani et al. [24]. This method assessed the efficiency of the extract for hydrogen donation in the formation of non-radicals in DPPH-H form [25]. In brief, 2.5 mL of 0.1 mM DPPH solution (dissolved in ethanol) was added to 0.5 mL of corn cob extract and mixed completely. After a 30-minute reaction of the mixture solution in darkness, the absorbance was measured at 517 nm. The percentage inhibition of DPPH radicals (% Inhibition) was determined using the following equation (1).

$$\% \text{ Inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100\% \quad (1)$$

Where $A_{control}$ was the absorbance of the DPPH solution with solvent, while A_{sample} was the absorbance of the DPPH solution with the field corn cob extract.

Subsequently, field corn cob extract powder from the optimal extraction conditions (maximum TPC and DPPH inhibition) was determined the concentration required to IC_{50}. The freeze-dried powder of field corn cob extract was evaluated for %DPPH inhibition at varying concentrations of 1.0, 2.0, 3.0, 4.0, and 5.0 mg/mL ($y = 0.191x + 2.5474$, $R^2 = 0.998$). The IC_{50} of the field corn cob extract was finally

compared with ascorbic acid as the standard at concentrations of 10, 20, 30, 40, and 50 µg/mL ($y = 1.7258x - 0.3293$, $R^2 = 0.9963$).

2.8 Statistical analysis

The experiment was performed in triplicate, and the results were reported as the mean ± standard deviation (SD). The data analysis used for the test was a paired t-Test for two samples for means, with a confidence level of $p < 0.05$ in Excel. When the p-value was greater than the alpha level of 0.05, there was no significant difference in the means of each sample.

3. Results and Discussion

The field corn cobs were extracted with 50% ethanol solvent by varying extraction times (30, 60, 120, 240, and 360 minutes). In this study, a binary ethanol-water mixture was selected as the solvent for the extraction of phenolic compounds. Table 1 shows that the TPC of the extract obtained from ethanol extraction increased significantly with longer extraction times, as indicated by different superscript letters ($p < 0.05$). The lowest TPC was observed at 30 minutes (155.5 ± 0.4 µg GAE/g CC), while the highest TPC was obtained at 360 minutes (221.9 ± 4.3 µg GAE/g CC). However, the TPC values at 240 and 360 minutes did not differ significantly, indicating that 240 minutes was the optimal extraction time for obtaining phenolic compounds from field corn cob using ethanol extraction. This trend suggests that extending the extraction time enhances the release or solubilization of phenolic compounds from the field corn cob matrix, but the effect plateaus at 240 minutes.

The results corresponded to the findings of Saewan et al. (2020) [26], which showed that TPC of coffee pulp significantly increased as extraction time extended from 1 to 3 hours, followed by a slight increase and stabilization during 4 to 5 hours. Extended extraction time improved the extraction

Table 1 Total phenolic content and antioxidant activity of field corn cob extract using 50% ethanol solvent.

Extraction time (minutes)	Total phenolic content (µgGAE/g CC)	DPPH radical scavenging (%)
30	155.5 ± 0.4^{a}	61.4 ± 0.3^{a}
60	185.4 ± 4.1^{b}	63.9 ± 1.7^{a}
120	203.4 ± 1.6^{c}	50.6 ± 0.4^{b}
240	214.9 ± 1.4^{d}	49.0 ± 0.6^{b,c}
360	221.9 ± 4.3^{d}	48.5 ± 0.8^{c}

Values are expressed as mean ± standard deviation, n = 3. The different letters indicated significant differences from one another. In the column of TPC, the letters ($^{a-c}$) = $p < 0.01$ and (^{d}) = $p < 0.05$. In the column of DPPH radical scavenging, the letters (^{a,b}) = $p < 0.01$ and (^{c}) = $p < 0.05$.

of phenolic compounds by allowing sufficient solute exposure to the solvent, thereby enhancing the recovery of phenolic compounds [27]. However, extending extraction time might raise the risk of phenolic oxidation [22], as shown in the results of DPPH radical scavenging activity. The percentage of DPPH radical inhibition increased from 61.4 ± 0.3% at 30 minutes to 63.9 ± 1.7% at 60 minutes. After that, when the extraction time increased to 120 minutes, the DPPH radical scavenging activity decreased, and this decreased significantly with extended extraction time. By 360 minutes, the activity declined to 48.5 ± 0.8%. This decrease may be attributed to the extended extraction leading to the decomposition, and the degradation of antioxidant compounds which reduced antioxidant activity [27]. Even though extending the extraction time increased TPC up to 240 minutes, the DPPH radical scavenging activity of the field corn cob extract peaked at 60 minutes. Therefore, the extraction time limit using ethanol was specified as 60 minutes with regarding the antioxidant activity was prior order.

Next, the UAE process utilized 37 kHz ultrasound combined with ethanol solvent for extraction at durations of 15, 30, and 60 minutes. Table 2 shows the TPC and

DPPH radical scavenging activity of field corn cob extract using UAE at various times. The results showed a correlation between extraction time, TPC, and antioxidant activity. The increased of extraction time led to a significant increase ($p < 0.05$) in both TPC and DPPH radical scavenging activity. The TPC increased from 159.4 ± 4.7 µg GAE/g CC at 15 minutes to 307.8 ± 1.3 µg GAE/g CC at 60 minutes. The DPPH radical scavenging activity increased from 78.8 ± 1.1% at 15 minutes to 85.1 ± 0.4% at 60 minutes. Consequently, extraction duration influenced both the phenolic yield and antioxidant activity obtained via the UAE method. The most effective extraction occurred at 60 minutes, which is consistent with the finding of Hernández et al. [28] regarding the extraction of phenolic compounds from red corn cobs. Typically, a longer extraction time in the UAE process could improve the efficiency of phenolic compound extraction [29]. Nonetheless, the long extraction time resulted in the degradation of phenolic compounds [30]. This elucidation was supported by the results of Shehata et al. [31], which demonstrated that a duration of 30-60 minutes was optimal for polyphenol extraction from orange peels utilizing UAE. An increased extraction duration of 70–80 minutes led to a decrease in the phenolic yield.

Table 2 Total phenolic content and antioxidant activity of field corn cob extract using ultrasound-assisted extraction at various times.

Extraction time (minutes)	Total phenolic content (µgGAE/g CC)	DPPH radical scavenging (%)
15	159.4 ± 4.7^{a}	78.8 ± 1.1^{a}
30	242.7 ± 0.7^{b}	81.3 ± 0.4^{b}
60	307.8 ± 1.3^{c}	85.1 ± 0.3^{c}

Values are expressed as mean ± standard deviation, n = 3. The different letters indicated significant differences from one another. In the column of TPC, the letters ($^{a-c}$) = $p < 0.01$. In the column of DPPH radical scavenging, the letters (^{a,c}) = $p < 0.05$ and (^{b}) = $p < 0.01$.

In addition, TPC of the extract from UAE for 15 minutes (159.4 ± 4.7 µg GAE/g CC) was close to that obtained from ethanol extraction for 30 minutes (155.5 ± 0.4 µg GAE/g CC). Extending the UAE time to 30 minutes, the extract had a higher TPC (242.7 ± 0.7 µg GAE/g CC) than the 360 minutes ethanol extraction (221.9 ± 4.3 µg GAE/g CC). The best result of TPC occurred at 60 minutes of UAE which was 307.8 ± 1.3 µg GAE/g CC. Additionally, the results of antioxidant activity, the extract from UAE exhibited greater activity than the extract from ethanol extraction. DPPH radical scavenging activity of the extracts from UAE for 15-60 minutes was in the range of 78.8-85.1%. Compared with the antioxidant activity of the extracts obtained from ethanol extraction, it ranged from 48.5 to 63.9%. The observed increase in TPC and antioxidant activity within a reduced duration indicates that UAE enhances the efficiency of the extraction process. It can be explained that high-frequency waves caused a disruption in the bonding of the solute-solvent mixture and the rupture of cellular membranes. When this happened, the cell broke and the solvent diffused throughout the cell plant, enabling extraction. Bioactive compounds, including antioxidants, were easier released into the solvent and resulted in yielding efficient extraction [32]. This method may be particularly beneficial for industrial applications where the extraction time is reduced, and the extract quality is better.

When the comparison of TPC and DPPH radical scavenging percentages from the two extraction methods at 60 minutes as illustrated in Figure 1, the results clearly demonstrate that UAE outperformed ethanol extraction alone in both TPC and antioxidant activity. The TPC of field corn cob extract from UAE (307.8 ± 1.3 µg GAE/g CC) was higher than from ethanol extraction (185.4 ± 4.1 µg GAE/g CC). This improvement is attributed to the ultrasonic waves disrupting cell walls and enhancing

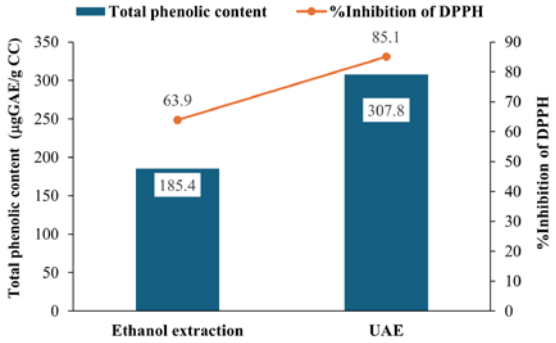

Figure 1 Total phenolic content and DPPH radical scavenging percentage of field corn cob extract using ethanol extraction and UAE at 60 minutes.

solvent penetration. Notably, UAE also enhanced antioxidant activity, with DPPH radical scavenging percentages reaching 85.1% at 60 minutes, outperforming conventional extraction (63.9%). This means that 50% ethanol, 37 kHz ultrasound waves, 60 minutes, and a temperature of 50°C are the best conditions for extracting phenolic compounds from field corn cobs. These results are like what other plant studies have found. A study by Almusallam et al. (2021) [33] also showed that the best UAE conditions were frequency 40 kHz, temperature 40.8 °C, duration 21.6 min, and ethanol concentration 50%. These conditions were able to get the most total phenolic content from date palm spikelets, which was 130.2 mg GAE. Furthermore, Nipornram et al. (2018) [19] revealed that UAE at 48 °C, 38.5 kHz, and 56.71 W for 40 minutes was more effective than maceration extraction at extracting phenolic compounds from mandarin peel.

Last, the field corn cob extract was produced under optimal conditions using UAE on a large scale and subsequently freeze-dried to assess the value of total phenolic content and antioxidant activity. The extract contained a total phenolic content of 1125.38 μg GAE/g CC. The antioxidant efficiency of field corn cob extract was analyzed and reported as the IC_{50} value in comparison to ascorbic acid. The IC_{50} value served as a crucial measure of the antioxidant strength of the extracts. A lower IC_{50} value indicated that the antioxidant activity was better [34]. The field corn cob extract exhibited an IC_{50} value of 2484.43 μg/mL (2.48 mg/mL) as shown in Figure 2(a), significantly higher than ascorbic acid, which had an IC_{50} value of 29.16 μg/mL as shown in Figure 2(b). These results indicated that although field corn cob extract exhibited antioxidant activity, it was less effective than ascorbic acid. Even so, it still showed potential as a source of natural antioxidants, similarly to other sources.

In comparing the IC_{50} values from this study with prior research in Table 3, the IC_{50} of field corn cob extract was lower than the 4.64 mg/mL observed for purple waxy corn cob obtained via the UAE method [35]. The polysaccharides from sweet corn cob polysaccharide-iron complexes exhibited IC_{50} values ranging from 5.01-8.34 mg/mL [36]. The IC_{50} value of field corn cob extract was slightly lower than that of the ethanolic extract of Pistachio (*Pistacia vera* L.) hull, recorded at 2.73 mg/mL, derived from microwave-assisted extraction [37]. The antioxidant activity of field corn cob extract outperformed that of purple waxy corn cob, sweet corn cob polysaccharide-iron (III) complex, and pistachio hull, yet was inferior to extracts

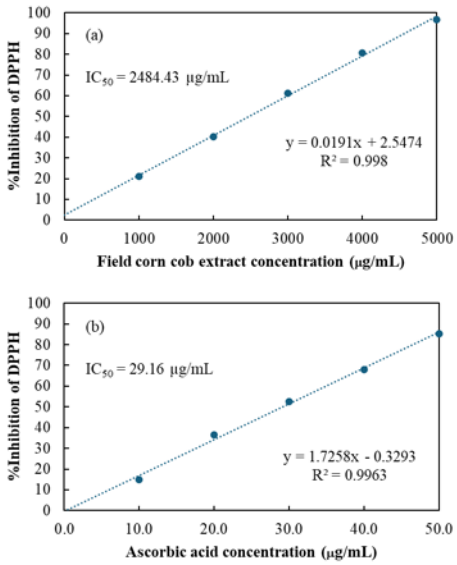

Figure 2 IC_{50} values of (a) field corn cob extract and (b) ascorbic acid, respectively.

Table 3 Comparison of the antioxidant activity of field corncob extract with that of other plants and methods.

Source of plant	Extraction method	IC_{50} value
Field corn cob	UAE	2484.43 μg/mL (2.48 mg/mL)
Purple waxy corn cob [35]	UAE	4.64 mg/mL
Sweet corn cob polysaccharides-iron complexes [36]	Synthesis with $FeCl_3$	5.01-8.34 mg/mL
Pistachio hull [37]	MAE	2.73 mg/mL
Corn cob extract [38]	SFE	0.6 mg/mL
Combination of purple waxy corn cob and pandan leave extract [39]	Water extraction	56.37 μg/mL

derived from advanced techniques, such as supercritical CO_2 extraction. However, IC_{50} of field corn cob extract was higher than 0.6 mg/mL of corn cob extract obtained from a supercritical CO_2 process utilizing a ternary green system of ethanol and water [38]. The combination of purple waxy corn cob and pandan leaf extract exhibits significant activity, as evidenced by an IC_{50} of 56.37 μg/mL [39]. These studies indicated that the source and extraction technique employed affected the antioxidant yield.

To get even more phenolic yield and antioxidant activity, the next experiment should focus on finding the best UAE parameters, such as frequency, ethanol concentration, and temperature. The optimized UAE method reduces extraction time and energy consumption, making it suitable for large-scale applications. Furthermore, enhancing the antioxidant activity involves combining it with other natural antioxidants. The findings support the sustainable utilization of field corn cobs as a valuable source of natural antioxidants. Exploring their applications expands in functional foods, nutraceuticals and cosmetics. This approach nutraceuticals and cometics potential for sustainable and practical applications in food cosmetics, and pharmaceuticals.

4. Conclusion

The application of UAE was a successful method in enhancing the extraction of phenolic compounds from field corn cobs. The UAE method resulted in increased extraction of phenolic compounds and demonstrated enhanced antioxidant activity, suggesting that it effectively improves the extraction of bioactive compounds from plants. This technique not only reduced extraction time but also increased the quantity and efficiency of antioxidant extraction, which is potentially advantageous for applications in the food, pharmaceutical, and cosmetic areas. The findings suggest that ultrasound-assisted extraction is a more effective technique for extracting antioxidants from corn cobs than conventional ethanol extraction. Even so, the antioxidant activity of the corn cob extract was still lower than that of ascorbic acid. This indicates that the extraction process requires enhancement and may yield better results when combined with other antioxidant-rich substances. This study demonstrates that field corn cobs can be repurposed as a valuable source of bioactive compounds for use in various industries, such as food, pharmaceuticals, and cosmetics.

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