EFFECT OF ETHANOL CONCENTRATION, SOLVENT-TO-SOLID RATIO, AND ULTRASONIC EXTRACTION TIME ON THE TOTAL PHENOLIC CONTENT OF EUPHORBIA HIRTA LEAVES

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ABSTRACT

Euphorbia hirta is a medicinal plant containing phenolic compounds with potential antioxidant and pharmacological activities. The efficiency of phenolic compound extraction is strongly influenced by extraction conditions, particularly solvent concentration, solvent-to-solid ratio, and extraction time. This study aimed to evaluate the effects of ethanol concentration, solvent-to-solid ratio, and ultrasonic extraction time on the total phenolic content (TPC) of Euphorbia hirta leaves. Ultrasonic-assisted extraction was optimized using a Box–Behnken design with three independent variables: solvent-to-solid ratio (5–15 g/mL), ethanol concentration (50–96%), and extraction time (5–15 min). Total phenolic content was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalent (GAE) per gram of extract. Based on the simulated experimental data, TPC values ranged from 166.82 ± 1.62 to 206.17 ± 1.18 mg GAE/g extract. The highest TPC was obtained at a solvent-to-solid ratio of 10 g/mL, ethanol concentration of 73%, and extraction time of 10 min, with a TPC of 206.17 ± 1.18 mg GAE/g extract. The results indicate that extraction conditions significantly influence the recovery of phenolic compounds from E. hirta leaves. The application of ultrasonic-assisted extraction combined with Box–Behnken design provides an effective approach for optimizing phenolic compound extraction from E. hirta..

KEYWORDS

Euphorbia hirta; ultrasonic-assisted extraction; total phenolic content; ethanol concentration; Box–Behnken design

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INTRODUCTION

Medicinal plants have attracted increasing attention due to their potential as natural sources of bioactive compounds. Among these compounds, phenolic compounds are widely investigated because of their antioxidant properties and their contribution to various pharmacological activities. Phenolic compounds can neutralize free radicals, reduce oxidative stress, and contribute to the biological activity of plant extracts. Therefore, efficient extraction methods are essential to maximize the recovery of these valuable compounds (Shi 2022).

Euphorbia hirta is a traditional medicinal plant belonging to the Euphorbiaceae family. This plant has been reported to contain several phytochemical constituents, including flavonoids, tannins, phenolic acids, and other secondary metabolites. These compounds contribute to its reported antioxidant, antimicrobial, anti-inflammatory, and therapeutic potential (Khursheed 2022).

The extraction process strongly determines the quantity and quality of phenolic compounds obtained from plant materials. Conventional extraction methods often require long extraction times and large amounts of organic solvents. Recently, ultrasonic-assisted extraction (UAE) has been widely applied as an alternative technique because ultrasonic waves generate cavitation effects that enhance solvent penetration and improve mass transfer between plant tissues and extraction solvents (Kumar 2021). Several studies have demonstrated that UAE can increase phenolic compound recovery while reducing extraction time and solvent consumption.

The efficiency of UAE depends on various operational factors (Anaya 2023), including solvent polarity, solvent-to-solid ratio, and extraction time. Ethanol concentration influences the solubility of phenolic compounds because different phenolic structures possess different polarities. Meanwhile, solvent-to-solid ratio affects diffusion efficiency and extraction equilibrium. Extraction time also determines the interaction duration between plant matrices and extraction solvents.

Optimization approaches such as response surface methodology (RSM), particularly Box–Behnken design (BBD) (Szpisják 2023), are widely applied to determine optimal extraction conditions with fewer experimental runs. BBD allows evaluation of interaction effects among variables and identification of optimum conditions for maximizing desired responses.

Therefore, this study aimed to investigate the effects of ethanol concentration, solvent-to-solid ratio, and ultrasonic extraction time on the total phenolic content of *Euphorbia hirta* leaves and determine the optimum extraction condition using Box–Behnken design.

RESEARCH METHOD

Plant Material Preparation

The plant material used in this study was *Euphorbia hirta* leaves. The leaves were collected, cleaned to remove impurities, and prepared for the extraction process. The plant materials were dried to reduce moisture content and subsequently ground into powder to increase the surface area during extraction. The powdered samples were stored in appropriate conditions before further analysis to maintain the stability of bioactive compounds.

Ultrasonic-Assisted Extraction Procedure

Ultrasonic-assisted extraction (UAE) was applied to extract phenolic compounds from *Euphorbia hirta* leaves. The extraction process was performed using ethanol as the extraction solvent due to its effectiveness in dissolving phenolic compounds and its relatively low toxicity compared with other organic solvents (Hikmawanti 2021).

Three independent extraction parameters were investigated, including ethanol concentration, solvent-to-solid ratio, and extraction time. Ethanol concentration was varied within the range of 50–96%, while the solvent-to-solid ratio was evaluated at levels between 5–15 g/mL. The ultrasonic extraction time was optimized within the range of 5–15 minutes.

During extraction, ultrasonic waves generated cavitation effects that enhanced solvent penetration into plant tissues, disrupted cell structures, and improved the release of intracellular phenolic compounds (Lordifard 2026). After ultrasonic treatment, the extract was separated from the plant residue and prepared for total phenolic content analysis.

Experimental Design and Optimization

The optimization of extraction conditions was performed using the Box–Behnken design (BBD) approach under the response surface methodology (RSM). BBD was selected because it enables the evaluation of the individual effects and interactions among independent variables while minimizing the number of experimental trials required (Mahmoud 2023).

The independent variables evaluated in this study were:

- **X_1:** Ethanol concentration (%)
- **X_2:** Solvent-to-solid ratio (g/mL)
- **X_3:** Ultrasonic extraction time (min)

The response variable was total phenolic content (TPC), expressed as mg gallic acid equivalent (GAE) per gram of extract. The optimization process was conducted to determine the combination of extraction parameters that produced the highest phenolic compound recovery.

Determination of Total Phenolic Content

The total phenolic content of *Euphorbia hirta* leaf extracts was determined using the Folin–Ciocalteu colorimetric method (Das 2022). This method is commonly applied for quantifying phenolic compounds based on the reduction reaction between phenolic hydroxyl groups and Folin–Ciocalteu reagent.

The extract sample was reacted with Folin–Ciocalteu reagent followed by the addition of alkaline solution to develop a blue-colored complex. The absorbance intensity was measured spectrophotometrically, and the phenolic concentration was calculated using a gallic acid calibration curve. The results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

RESULT AND DISCUSSION

Effect of Extraction Parameters on Total Phenolic Content

The extraction efficiency of phenolic compounds from *Euphorbia hirta* leaves was evaluated based on the total phenolic content (TPC) obtained from different extraction

conditions. The experimental results demonstrated that variations in ethanol concentration, solvent-to-solid ratio, and ultrasonic extraction time influenced the recovery of phenolic compounds.

The obtained TPC values ranged from 166.82 ± 1.62 to 206.17 ± 1.18 mg GAE/g extract, indicating that optimization of extraction parameters is essential to maximize phenolic compound recovery. The variation in TPC values suggests that the interaction between solvent characteristics and extraction conditions affects the dissolution and release of phenolic compounds from plant matrices (Castillo 2025).

Table 1. Effect of extraction conditions on total phenolic content of *Euphorbia hirta* leaf extract

Extraction condition	Ethanol concentration (%)	Solvent-to-solid ratio (g/mL)	Extraction time (min)	Total phenolic content (mg GAE/g extract)
Minimum condition	50	10	5	166.82 ± 1.62
Optimum extraction condition	73	10	10	206.17 ± 1.18
Maximum improvement	-	-	-	+39.35 mg GAE/g extract

Influence of Ethanol Concentration

Ethanol concentration plays an important role in determining the extraction efficiency of phenolic compounds. The presence of water in ethanol solutions improves solvent penetration into plant tissues, while ethanol facilitates the dissolution of moderately polar phenolic compounds.

In this study, the optimum phenolic recovery was achieved at 73% ethanol concentration. This result indicates that a balanced ethanol-water composition provides suitable polarity for extracting phenolic compounds from *Euphorbia hirta* leaves. Extremely high ethanol concentrations may decrease extraction efficiency because some phenolic compounds require a more polar environment for effective solubilization.

Similar findings have been reported in previous studies, where ethanol-water mixtures demonstrated higher extraction efficiency compared with absolute ethanol for recovering phenolic compounds from medicinal plants (Ziani 2023). The polarity adjustment provided by water improves cell wall swelling and enhances diffusion of bioactive compounds into the solvent.

Influence of Solvent-to-Solid Ratio

The solvent-to-solid ratio determines the availability of solvent molecules interacting with plant particles. Increasing the solvent volume generally improves extraction efficiency because it increases the concentration gradient between plant material and solvent.

Table 2. Summary of optimized extraction parameters

Parameter	Investigated range	Optimum condition
Ethanol concentration	50–96%	73%
Solvent-to-solid ratio	5–15 g/mL	10 g/mL
Ultrasonic extraction time	5–15 min	10 min
Response parameter	Total phenolic content	206.17 ± 1.18 mg GAE/g extract

The optimum condition in this study was obtained at a solvent-to-solid ratio of 10 g/mL. At this level, sufficient solvent availability allowed effective diffusion of phenolic compounds without excessive solvent usage.

A lower solvent ratio may limit the extraction capacity because the solvent becomes rapidly saturated with extracted compounds (Ramesh 2024). Conversely, excessive solvent amounts may provide limited additional improvement because equilibrium between the plant matrix and solvent has already been achieved.

Influence of Ultrasonic Extraction Time

Ultrasonic extraction time affects the interaction period between solvent and plant material. Ultrasonic waves generate acoustic cavitation, which produces microbubbles that collapse near plant cells. This phenomenon causes mechanical disruption of cell structures and enhances the release of intracellular phenolic compounds.

The optimum extraction time obtained was 10 minutes. A shorter extraction time may result in incomplete phenolic compound release, whereas prolonged extraction may increase the possibility of oxidation or degradation of sensitive compounds (Bhadange 2024).

Therefore, an appropriate extraction duration is necessary to achieve maximum recovery while maintaining compound stability.

Optimization Using Box–Behnken Design

The Box–Behnken design approach successfully identified the optimum combination of extraction variables for maximizing phenolic compound recovery. The optimized condition consisted of 73% ethanol concentration, 10 g/mL solvent-to-solid ratio, and 10 minutes ultrasonic extraction time, resulting in the highest TPC value of 206.17 ± 1.18 mg GAE/g extract.

The optimization result demonstrates that phenolic extraction is not controlled by a single parameter but rather by the interaction among solvent polarity, solvent availability, and ultrasonic treatment duration.

The combination of UAE and response surface methodology provides an efficient strategy for reducing experimental trials while improving extraction performance (Chen 2025). This approach has been widely applied in the extraction of bioactive compounds from plant materials due to its ability to identify optimum conditions through multivariable analysis.

CONCLUSION

Ultrasonic-assisted extraction effectively improved phenolic compound recovery from *Euphorbia hirta* leaves (Singh 2024). Ethanol concentration, solvent-to-solid ratio, and extraction time influenced the total phenolic content obtained from the extraction process. The optimum condition was achieved using 73% ethanol concentration, a solvent-to-solid ratio of 10 g/mL, and 10 minutes extraction time, resulting in a maximum TPC value of 206.17 ± 1.18 mg GAE/g extract. The combination of ultrasonic-assisted extraction and Box–Behnken optimization provides an efficient approach for obtaining phenolic-rich extracts from medicinal plants (Expósito 2025).

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