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Optimisation of ultrasound-assisted extraction of ethyl *p*-methoxycinnamate from *Kaemperia galanga* L. rhizomes

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Abstract:

Ultrasound-assisted extraction has become a prevalent technique for isolating various natural bioactive compounds. This study examines the influence of ultrasonic power, extraction temperature, and extraction time on the yield of ethyl *p*-methoxycinnamate (EPMC) from Kaemperia *galanga* rhizomes. The content of EPMC in the residue and the purity of the isolated EPMC were quantified using the gas chromatography - mass spectrometry (GC/MS) method. Response surface methodology (RSM) identified the optimal extraction conditions as follows: ultrasonic power of 100 W, extraction temperature of 56°C, and extraction time of 34 minutes. Under these conditions, the maximum EPMC extraction efficiency was 2.93%. Subsequently, ultrasound-assisted extraction at these conditions, combined with recrystallisation, was employed to isolate EPMC with a high purity exceeding 95% and an isolation efficiency of approximately 2.53% relative to the dried raw material. The purification efficiency of EPMC from the extract was approximately 82.9%. The isolation procedure proved to be stable and reproducible, marking the first report on the ultrasound-assisted extraction of EPMC from *Kaemperia galanga* rhizomes.

Keywords: **ethyl *p*-methoxycinnamate, *Kaemperia galanga* L., response surface methodology, ultrasound-assisted extraction.**

Classification numbers: **2.2, 3.3, 3.5**

1. Introduction

Kaemperia galanga L., an important medicinal plant in the Zingiberaceae family, is widely distributed across Vietnam, China, Japan, India, Sudan, Bangladesh, Sri Lanka, and other Southeast Asian countries including Indonesia, Laos, Malaysia, and Thailand [1]. Commonly known as "Địa liền" in Vietnam, *K. galanga* is traditionally used to treat stomach diseases, digestive disorders, and arthritis [2]. Additionally, it features numerous Ayurvedic remedies for managing hypertension, diabetes, respiratory conditions such as asthma and cough, rheumatic ailments, inflammation, colds, and diarrhoea [3]. Ethyl *p*-methoxycinnamate (EPMC), a key chemical constituent of *K. galanga*, is purported to underlie its pharmacological efficacy [4]. Previous studies have explored several extraction methods for EPMC, such as steam distillation, Soxhlet extraction, supercritical CO_2 extraction, and maceration. These methods, particularly steam distillation, Soxhlet extraction, and maceration, typically yield low extraction efficiencies of approximately 0.67-1.99%, varying with the solvent used [5-7]. In contrast, supercritical CO_2 extraction has been shown to enhance the extraction efficiency to about 2.5%, albeit requiring costly and complex apparatus [7].

Ultrasound-assisted extraction, or sonication, leverages ultrasonic wave energy to expedite the dissolution and diffusion of bioactive compounds and enhance heat transfer, thereby improving extraction efficiency. This method offers the advantages of reduced solvent and energy usage, alongside lower temperatures and shorter extraction durations [8].

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Response surface methodology (RSM) is a suite of statistical and mathematical tools crucial for modelling and optimising the relationship between multiple input variables and the desired output - here, the extraction efficiency of EPMC. This study details the optimisation of ultrasound-assisted extraction parameters for EPMC from *K. galanga* rhizomes using RSM, highlighting the collective impact of ultrasonic power, extraction temperature, and extraction time on the extraction efficiency [9].

In the present study, we report the results of optimising ultrasound-assisted extraction of EPMC from rhizomes using RSM. This technique is an effective tool for process optimisation, particularly when independent variables such as ultrasonic power, extraction temperature, and extraction time collectively influence the desired outcome-namely, EPMC extraction efficiency.

2. Materials and methods

2.1. Plant material

Dried *K. galanga* rhizomes were procured from households in Bac Giang province, Vietnam, in December 2023. The voucher specimens, labelled KG2023, have been deposited at the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST).

2.2. Extraction

The powdered dried *K. galanga* rhizomes were extracted using 96% (v/v) ethanol in an ultrasonic bath (Elmasonic S80H, Germany, frequency 37 kHz). Ethanol 96%, chosen for its safety, affordability, and environmental friendliness, effectively dissolves EPMC. For each extraction, exactly 2 g of *K. galanga* powder was mixed with 20 ml of ethanol (ratio 1:10). The optimal extraction conditions, including ultrasonic power, extraction temperature, and extraction time, were determined. Following extraction, the solution was cooled to room temperature and centrifuged at 3500 rpm for 10 minutes using a Hermle Z206A centrifuge (Germany) to collect the extract. The solvent was then evaporated under reduced pressure at 50°C for 5 minutes using a rotary evaporator to yield the dried residue, which was subsequently weighed and analysed by GC/MS. Each experiment was replicated three times to establish an average value. EPMC extraction efficiency was calculated using the formula (1):

$$\text{EPMC extraction efficiency (\%)} = \frac{(\text{Yield of extraction} \times \text{Content of EPMC in the residue})}{100} \quad (1)$$

The extraction efficiency and the content of EPMC in the residue were key metrics for evaluating the effectiveness of the extraction process.

2.2.1. Effect of ultrasonic power on extraction

The impact of ultrasonic power on extraction was assessed at five levels: 0, 70, 85, 100, and 115 W. For each level, 2 g of *K. galanga* powder was extracted with 20 ml of ethanol and ultrasonicated for 30 minutes at 50°C. For comparison, a non-ultrasonicated sample was extracted under the same temperature for 8 hours.

2.2.2. Effect of temperature on extraction

The influence of extraction temperature was evaluated at four levels: room temperature, 40, 50, and 60°C. For these experiments, 2 g of *K. galanga* powder was ultrasonicated in 20 ml of ethanol for 30 minutes at the chosen ultrasonic power across the investigated temperatures.

2.2.3. Effect of time on extraction

The effect of extraction time was explored at four durations: 10, 20, 30, and 40 minutes, using 2 g of *K. galanga* powder extracted with 20 ml of ethanol at the previously determined optimal ultrasonic power and extraction temperature.

2.3. Optimisation of ultrasound-assisted extraction of ethyl p-methoxycinnamate from K. galanga rhizomes using response surface methodology

In this article, we detail the optimisation of ultrasound-assisted extraction of EPMC from *K. galanga* rhizomes using RSM. Employing Minitab Software v.21.3, a three-level, three-factorial Box-Behnken design was implemented to determine the best conditions for ultrasonic power (W), extraction temperature (°C), and extraction time (min). The independent variables selected were based on preliminary experiments, and their ranges and levels are specified in Table 1.

Table 1. Independent variables and the factor levels of the experiment.

Independent variables	Factor levels		
	-1	*0*	*1*
Ultrasonic power (W)	70	85	100
Extraction temperature (°C)	40	50	60
Extraction time (min)	20	30	40

The experimental design consisted of 15 points, including three replicated experiments at the central point, executed in a random order as shown in Table 2.

Table 2. Box-Behnken design and experimental results.

Standard order	Run order	Ultrasonic conditions			EPMC extraction efficiency (%)
		Ultrasonic power (W)	*Extraction temperature (°C)*	*Extraction time (min)*	
14	1	0	0	0	2.86
6	2	1	0	-1	2.82
12	3	0	1	1	2.84
1	4	-1	-1	0	2.68
7	5	-1	0	1	2.76
15	6	0	0	0	2.87
9	7	0	-1	-1	2.69
11	8	0	-1	1	2.77
8	9	1	0	1	2.91
13	10	0	0	0	2.86
2	11	1	-1	0	2.82
3	12	-1	1	0	2.77
10	13	0	1	-1	2.79
5	14	-1	0	-1	2.64
4	15	1	1	0	2.92

A second-order polynomial equation was utilised to model the relationship between the variables and the EPMC extraction efficiency as seen in Eq. (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (2)$$

where X_1 is the ultrasonic power, X_2 is the extraction temperature, X_3 is the extraction time, β_0 is the constant term, β_1, β_2, β_3 are the linear regression coefficients, β_{12}, β_{13}, β_{23} are the interaction regression coefficients, and β_{11}, β_{22}, β_{33} are the quadratic regression coefficients.

2.4. Isolation of ethyl p-methoxycinnamate

The ultrasound-assisted extraction method, under optimised conditions, was employed to extract EPMC from *K. galanga* powder. This was followed by the recrystallisation process to purify EPMC from the resulting extract. A total of 2.0 kg of *K. galanga* powder was processed using 20 litres of ethanol 96% in a 50-litre-scale ultrasonic extraction machine (produced by Envitech, Vietnam) set to the optimised conditions. Post-extraction, the ethanol solvent was evaporated using a vacuum evaporator, yielding 500 ml of concentrated extract. This extract was then cooled at 3-5°C for 20 hours. After removing the supernatant, white crystalline EPMC was obtained through double recrystallisation using ethanol 96%. The purity of the isolated EPMC was verified using the GC/MS method.

2.5. Quantification of ethyl p-methoxycinnamate by gas chromatography - mass spectrometry

Analytical equipment and conditions: GC/MS analysis was conducted using a HP6890 gas chromatograph equipped with a 20 HP-5MS column (30 m x 0.25 mm x 0.25 µm) and an MSD 5973N Agilent detector. Helium was the carrier gas at a flow rate of 1.0 ml/min. The temperature programme started at 60°C, increased to 160°C at 20°C/min, then to 200°C at 4°C/min, and finally to 240°C at 20°C/min, where it was held for 1 minute. The injection volume was 1µl. EPMC was detected and identified using the Wiley275 spectral library.

Sample preparation: Extract sample: 250 mg of the dried extracted residue was dissolved in 100 ml of *n*-hexane, and the solution was filtered (pore size 0.45 µm) before analysis; EPMC sample: 1.0 mg of EPMC isolated from *K. galanga* rhizomes was dissolved in 1.0 ml of *n*-hexane. The obtained solution was filtered (pore size of 0.45 µm) before analysis.

Standard curve: Standard EPMC 99.8% was purchased from Sigma-Aldrich. EPMC quantitative standard curve was built based on the concentration range of 200, 400, 600, 800, and 1000 ppm using Chemstation software.

2.6. Statistical analysis

Data were processed using Excel software. Results were presented as mean±SD (standard deviation). A one-way ANOVA was conducted for statistical comparison between different groups, with $p<0.05$ considered statistically significant.

3. Results and discussion

3.1. Quantitative analysis of ethyl p-methoxycinnamate by gas chromatography - mass spectrometry

EPMC was quantitatively analysed under selected conditions, detected at a retention time of 11.74 minutes (Fig. 1). The molecular weight was confirmed as 206 with characteristic fragments at 206, 161, and 134 m/z (Fig. 2). The quantitative standard curve, y=18800x-998000 with R^2=0.999, was utilised for quantification of EPMC in the extracted residue and the isolated purity.

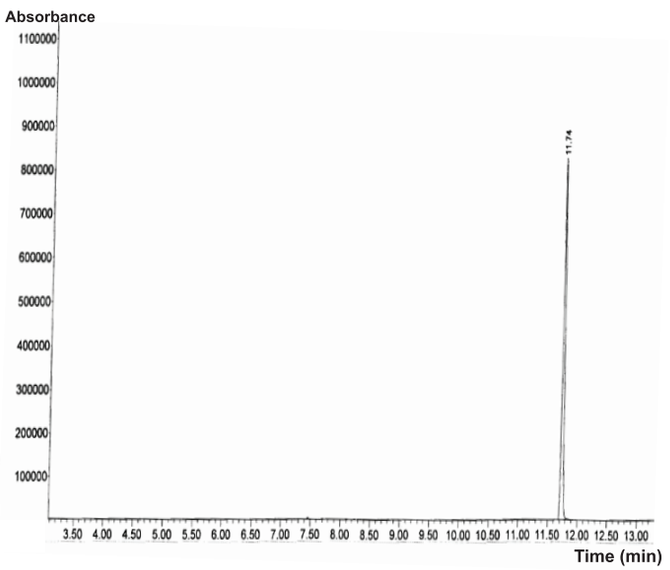

Fig. 1. Gas chromatography - mass spectrometry chromatogram of ethyl p-methoxycinnamate.

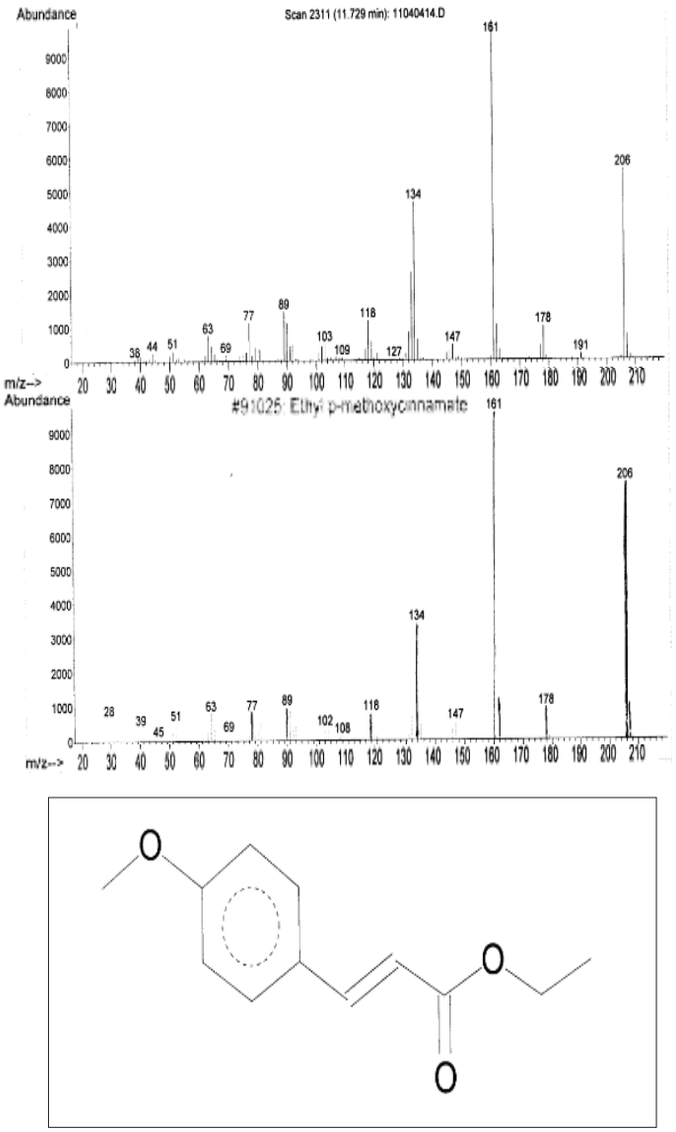

Fig. 2. Mass spectrometry spectra and structure of ethyl *p*-methoxycinnamate.

3.2. Effect of ultrasonic power on extraction

Table 3 illustrates the influence of ultrasonic power on EPMC extraction efficiency and residue content. Ultrasound promotes the breakdown of cell structures, enhancing the release of active ingredients compared to non-ultrasonic methods. Results indicated that with an ultrasonic power of 70 W, the extraction efficiency of EPMC was 2.48%, which was 1.13 times higher than non-ultrasonic extraction (2.18%) [10]. Incremental increases in ultrasonic power to 85 W and 100 W raised the extraction efficiency further to 2.76 and 2.91%, respectively, representing increases of 1.27 and 1.33 times over non-ultrasonic methods. However, increasing the ultrasonic power to 115W did not significantly enhance the extraction efficiency compared to 100 W ($p>0.05$).

Table 3. Effect of ultrasonic power on extraction.

No.	Ultrasonic power (W)	The weight of the extracted residue (mg)	Yield of extraction (%)	Content of EPMC in the residue (%)	EPMC extraction efficiency (%)
1	0	285.2±4.9	14.26±0.09	15.29±0.06	2.18±0.03
2	70	296.8±3.6	14.84±0.12	16.73±0.14	2.48±0.02
3	85	311.3±5.2	15.57±0.08	17.76±0.17	2.76±0.05
4	**100**	**321.8±4.7**	**16.09±0.10**	**18.08±0.09**	**2.91±0.06**
5	115	328.7±6.1	16.44±0.15	17.70±0.13	2.91±0.08

The results further demonstrated that an increase in ultrasonic power enhanced the content of EPMC in the extracted residue, achieving a peak content of 18.08% at 100 W. However, when the ultrasonic power was elevated to 115 W, the content of EPMC diminished to 17.70% ($p<0.05$). This reduction is likely due to excessive cellular disruption at higher ultrasonic powers, which facilitates the release of additional impurities.

3.3. Effect of temperature on extraction

Temperature is a crucial factor that not only influences the extraction efficiency but also affects the biological activity of the extract or its active components. A reduction in solution viscosity, an increase in the solvent's permeation into the cells, and enhanced extraction efficiency are all benefits of higher temperatures. However, excessively high temperatures can escalate costs and potentially degrade the quality of the extract, as some bioactive compounds are sensitive to heat. It is essential to determine the optimal extraction temperature for extracting EPMC from *K. galanga* rhizomes.

As indicated in Table 4, increasing the extraction temperature from room temperature to 40°C and 50°C significantly enhanced the extraction of EPMC ($p<0.05$). The optimal figures, both for EPMC extraction efficiency and content in the extracted residue, were recorded at 50°C, reaching 2.91 and 18.09%, respectively. Nevertheless, raising the temperature further to 60°C did not improve the extraction efficiency of EPMC compared to 50°C ($p>0.05$), and the content of EPMC in the residue decreased to 17.71% ($p<0.05$), likely due to the higher temperature dissolving other impurities more readily, thereby reducing the EPMC content in the residue.

Table 4. Effect of temperature on extraction.

No.	Ultrasonic temperature (°C)	The weight of the extracted residue (mg)	Yield of extraction (%)	Content of EPMC in the residue (%)	EPMC extraction efficiency (%)
1	room temperature	274.3±3.9	13.72±0.12	17.64±0.09	2.42±0.04
2	40	292.9±4.7	14.65±0.16	17.96±0.14	2.63±0.03
3	**50**	**321.5±4.9**	**16.15±0.11**	**18.09±0.15**	**2.91±0.05**
4	60	329.2±5.3	16.46±0.09	17.71±0.10	2.91±0.02

3.4. Effect of time on extraction

Table 5 shows the impact of extraction time on both the EPMC extraction efficiency and the content of EPMC in the residue. Results indicate that increasing the extraction time from 10 to 20 and then 30 minutes resulted in progressively higher EPMC extraction efficiencies, from 2.45 to 2.69% and 2.92%, respectively ($p<0.05$). The content of EPMC in the residue similarly increased, reaching a peak at 18.10% at 30 minutes ($p<0.05$). However, extending the extraction time to 40 minutes slightly decreased both the extraction efficiency and the EPMC content compared to 30 minutes ($p<0.05$).

Table 5. Effect of time on extraction.

No.	Ultrasonic time (min)	The weight of the extracted residue (mg)	Yield of extraction (%)	Content of EPMC in the residue (%)	EPMC extraction efficiency (%)
1	10	289.4±5.9	14.47±0.09	16.96±0.13	2.45±0.02
2	20	305.7±4.5	15.29±0.12	17.59±0.19	2.69±0.04
3	**30**	**322.5±5.1**	**16.10±0.14**	**18.10±0.12**	**2.92±0.05**
4	40	334.2±6.3	16.71±0.18	17.43±0.10	2.91±0.06

Table 6. Analysis of variance for ethyl *p*-methoxycinnamate extraction efficiency.

Source	Degrees of freedom (DF)	Adjusted sums of squares (Adj SS)	Adjusted mean squares (Adj MS)	F-value	p-value
Model	9	0.095483	0.010609	47.50	<0.001
Linear	3	0.078700	0.026233	117.46	<0.001
Power	1	0.048050	0.048050	215.15	<0.001
Temp.	1	0.016200	0.016200	72.54	<0.001
Time	1	0.014450	0.014450	64.70	<0.001
Square	3	0.016308	0.005436	24.34	0.002
Power *Power	1	0.002878	0.002878	12.88	0.016
Temp. *Temp.	1	0.005308	0.005308	23.77	0.005
Time *Time	1	0.010339	0.010339	46.29	0.001
2-Way Interaction	3	0.000475	0.000158	0.71	0.587
Power *Temp.	1	0.000025	0.000025	0.11	0.752
Power *Time	1	0.000225	0.000225	1.01	0.362
Temp. *Time	1	0.000225	0.000225	1.01	0.362
Error	5	0.001117	0.000223		
Lack-of-Fit	3	0.001050	0.000350	10.50	0.088
Pure Error	2	0.000067	0.000033		
Total	14				

The possible explanation for the decrease in EPMC content with prolonged extraction times under ultrasound might be the generation of free radicals, chemical reactions, oxidation, and thermal decomposition reactions [11, 12], which could lead to partial decomposition of EPMC. Another potential reason is that extended extraction periods facilitate the dissolution of macromolecules and impurities other than the target compound, EPMC.

3.5. Optimisation of extraction process using response surface methodology

The EPMC extraction efficiency was modelled using a second-order polynomial response surface model. Regression analysis and ANOVA were employed to fit the model and to assess the statistical significance of the terms, as shown in Table 6.

The p-values served as indicators of the significance of each coefficient; a smaller p-value implies greater significance. Coefficients with p-values less than 0.05 were considered significant. The F-value is a statistical test used to determine the association between the term and the response, which informs the calculation of the p-value. High F-values signify the significance of the term or model. Two-way interactions showed larger p-values (p-value>0.05), indicating low significance to the regression model, as predicted by the Box-Behnken design. The variables of ultrasonic power, temperature, and time demonstrated high significance in the model, evidenced by their very low p-values (p-value <0.001) [13]. The response plots for EPMC extraction efficiency versus the three different input variables, correlated two by two, are presented in Figs. 3-5.

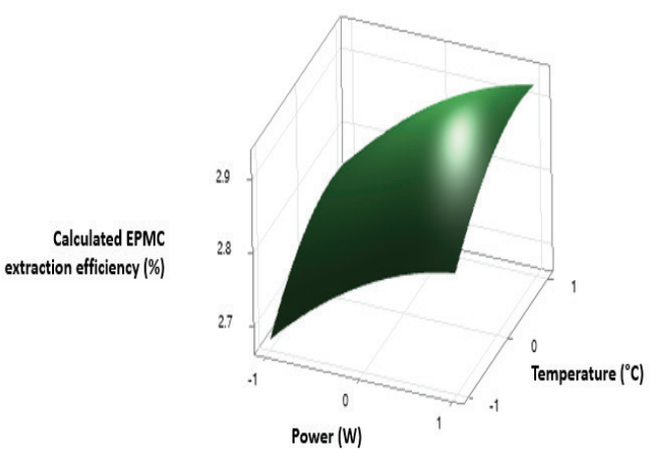

Fig. 3. Ethyl *p*-methoxycinnamate extraction efficiency vs. power and temperature.

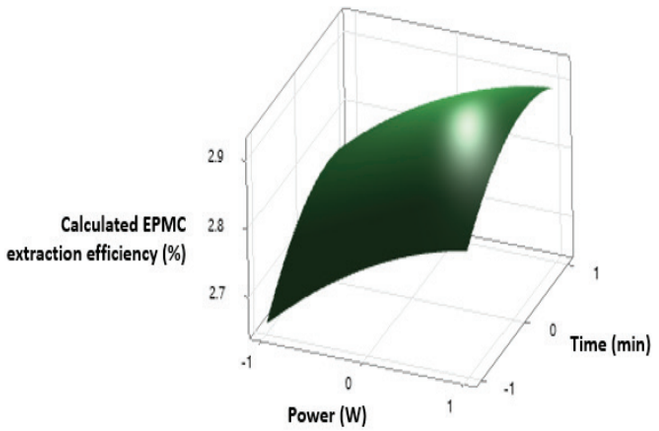

Fig. 4. Ethyl *p*-methoxycinnamate extraction efficiency vs. power and time.

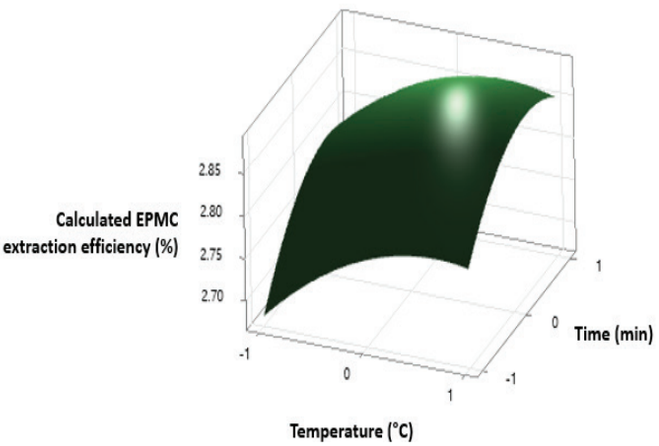

Fig. 5. Ethyl *p*-methoxycinnamate extraction efficiency vs. temperature and time.

The analysis of the response surface model for EPMC extraction efficiency was verified by measuring the R-squared coefficient (Table 7).

Table 7. Model summary.

	S	R-sq	R-sq (adj)	R-sq (pred.)
EPMC extraction efficiency	0.0149443	98.84%	96.76%	82.45%

where S is the standard deviation of the distance between the data values and the fitted values; R-sq is the coefficient of determination .

The coefficient of determination (R-sq) value was 98.84%, showing a strong correlation between the experimental and predicted data at a 95% confidence level. The quadratic model for predicting the optimal point was expressed by the following second-order polynomial equation (3):

$$EPMC\ extraction\ efficiency = 2.86333 + 0.07750X_1 + 0.04500X_2 + 0.04250X_3 + 0.00250X_1X_2 - 0.00750X_1X_3 - 0.00750X_2X_3 - 0.02792X_1^2 - 0.03793X_2^2 - 0.05292X_3^2 \quad (3)$$

The equation, after reducing the insignificant terms of the quadratic model, becomes (4):

$$EPMC\ extraction\ efficiency = 2.86333 + 0.07750X_1 + 0.04500X_2 + 0.04250X_3 + -0.02792X_1^2 - 0.03793X_2^2 - 0.05292X_3^2 \quad (4)$$

where X_1: Ultrasonic power; X_2: Extraction temperature; X_3: Extraction time.

The model indicated that the linear effects of ultrasonic power and extraction temperature had the most significant impact on EPMC extraction efficiency.

These data were used to select the optimal extraction conditions to maximise EPMC extraction efficiency, which achieved a maximum of 2.93% under the following conditions: ultrasonic power of 100 W, extraction temperature of 56°C, and extraction time of 34 minutes, with desirability very close to 1.

3.6. Isolation of ethyl p-methoxycinnamate

The optimised extraction conditions (100 W power, 56°C temperature, and 34 minutes time) were applied to perform three batches of EPMC isolation from *K. galanga* rhizomes (2 kg raw material per batch). The results are shown in Table 8.

Table 8. Results of ethyl *p*-methoxycinnamate isolation from *K. galanga* rhizomes.

Batch	Dried *K. galanga* powder (kg)	Content of EPMC in the extracted residue (%)	EPMC extraction efficiency (%)	Isolated EPMC (g)	EPMC isolation efficiency (%)	Purity of EPMC (%)
1	2.0	18.10	2.92	50.6	2.53	95.94
2	2.0	18.09	2.93	49.8	2.49	96.02
3	2.0	18.08	2.93	51.1	2.56	95.89
Mean	**2.0**	**18.09**	**2.93**	**50.5**	**2.53**	**95.95**

The average isolation efficiency of EPMC from *K. galanga* rhizomes using the ultrasound-assisted extraction method was 2.53%, significantly higher than previously reported methods such as steam distillation, Soxhlet extraction, and maceration (0.67-1.99%). Previous reports use organic solvents like *n*-hexane or ethyl acetate for extracting and purifying EPMC [5, 6] while our isolation process of EPMC just utilises ethanol, a common, safe, and inexpensive solvent. Notably, the ultrasound-assisted extraction method provided high isolation efficiency, comparable to the supercritical CO_2 extraction method [7], but with less time and without requiring expensive and complex equipment. The EPMC isolated had a high purity of over 95%. The purification efficiency of EPMC from the extract was approximately 82.9%. The process was stable

and repeatable, highlighting the effectiveness of response surface methodology to optimise EPMC extraction from *K. galanga* rhizomes (ultrasonic power of 100 W, extraction temperature of 56°C, and extraction time of 34 minutes) thereby increasing the EPMC extraction efficiency from 2.91 to 2.93% compared to previously optimised conditions. This approach has been widely applied to optimise extraction conditions, improving efficiency and product quality, saving time, costs, and reducing solvent volumes. Similar benefits have been reported in previous studies concerning the ultrasound-assisted extraction of oil from hazelnuts [14-17].

4. Conclusions

In summary, the extraction and isolation of EPMC from *K. galanga* rhizomes using the ultrasound-assisted extraction method have been comprehensively studied. Optimal conditions were identified using response surface methodology, resulting in high purity EPMC using a recrystallisation technique. This represents the first report of the ultrasound-assisted extraction and process optimisation of EPMC from *K. galanga* rhizomes.

CRediT author statement

Do Thi Dinh: Conceptualisation, Methodology, Writing - Original draft preparation, Structuring; Ngo Thi Phuong: Writing - Original draft preparation, Data analysis, Formatting; Ta Thi Thao: Writing - Original draft preparation, Data analysis; Do Thi Quynh An: Data analysis; Pham Thi Nhung: Methodology, Data collection; Nguyen Thai An: Writing - Reviewing and Editing; Le Minh Ha: Writing - Reviewing and Editing, Project administration.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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