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Cassia fistula L. fruit: Optimal conditions for total polyphenol extraction

Do Viet Phuong, Nguyen Ngoc Thuan*

Industrial University of Ho Chi Minh City, Ho Chi Minh, Vietnam

* e-mail: nguyenngocthuan@iuh.edu.vn

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Abstract:
This study optimized the polyphenol extraction conditions for *Cassia fistula* L., commonly known as the Osaka tree. Its fruit contains a complex of valuable natural compounds, which remain underexploited in Vietnam and other countries where Osaka trees grow in abundance.
Methods employed included phytochemical screening, determination of total phenolic and moisture content, preliminary factor screening, and optimization using Response Surface Methodology (RSM) with a Box-Behnken design, followed by statistical analysis using t-tests, ANOVA, and Tukey's HSD post-hoc test.
Most previous studies focused on testing and evaluating individual extraction parameters, such as temperature, solid-to-solvent ratio, and time. This study comprehensively integrated these factors into a unified optimization model. Based on the design of preliminary diagnostic experiments, the solid-to-solvent ratios ranged from 1:10 to 1:50 (w/v), the extraction temperature was 30–70°C, and the extraction time ranged from 5 to 30 min. The Box-Behnken model combined with response surface methodology made it possible to optimize multiple factors simultaneously. The total polyphenol content reached its highest value of 10.64 mg GAE/g DW under the following optimal conditions: 50°C; 21.15 min extraction time; 1:31.69 (w/v) solid-to-solvent ratio. The model demonstrated high reliability ($R^2 = 0.94$), confirming its industrial potential.
The results obtained open up new prospects for sustainable exploitation of natural polyphenols from *C. fistula* in functional foods and pharmaceuticals.

Keywords: *Cassia fistula* L., Osaka tree, bioactive compounds, extraction, optimization, response surface methodology, Box-Behnken model

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INTRODUCTION

Bioactive compounds derived from plants have been of interest in recent years because they are not only a source of food but also a valuable source of pharmaceuticals. In this context, indigenous plant species often become the focus of research due to their biodiversity and medicinal applications [1–4]. *Cassia fistula* L. is commonly known as the Osaka tree, Indian laburnum, or golden shower tree (*Fabaceae*). This plant stands out for its bright yellow inflorescences and is highly valued for its medicinal properties. It contains a cluster of bioactive compounds with antibacterial, insecticidal, and antioxidant properties [5, 6].

Cassia fistula L. fruit has a unique elongated cylindrical shape and turns dark brown when ripe. Beside its aesthetic value, the pod-like fruit has long been appreciated for its powerful laxative properties, as well as for its antibacterial, anti-inflammatory, neuroprotective, and cardioprotective effects [7]. However, its industrial use requires more scientific studies to verify its medicinal profile. Recent phytochemical studies demonstrated that Osaka fruits are rich in polyphenols, flavonoids, anthraquinones, and anthraquinone glycoside derivatives [8, 9]. These compounds exhibit strong antioxidant activity, which can protect cells from free radical damage, thus preventing such serious chronic diseases as diabetes, cancer, and neurodegenerative disorders. These findings open up many new application prospects for the functional food industry, pharmaceuticals, and cosmetics [10].

In Vietnam, Osaka trees are widely distributed in humid and tropical environments, but their medicinal potential has not been fully exploited [11]. Most applications are still limited to folk remedies. This shows a huge gap between the abundance of resources and their effective application. To maximize the exploitation pro-

cess and enhance the economic value of this promising plant, scientists and affiliated companies must conduct more in-depth investigations [12]. One of the most promising directions is to optimize the extraction of bioactive compounds, especially total phenolics, which are known for their anti-inflammatory and cardioprotective effects [13].

The extraction of natural compounds from plants is a complex process because the yield depends on many factors. In this research, we combined the response surface methodology with the Box-Behnken experimental design to reduce the number of experiments, thereby reducing time and cost compared to the traditional trial and error method. In addition, this method also helps understand the multidimensional correlation between different factors in the extraction process [14]. Furthermore, advanced techniques, such as microwave-assisted extraction or ultrasound-assisted extraction can further improve the phenolic recovery [15].

This study had two main objectives:

– to evaluate the effects of such technological factors as ethanol solvent concentration, temperature, and extraction time on the total phenolic yield;

– to use the response surface methodology to develop an accurate mathematical model that would make it possible to optimize the polyphenol extraction process while minimizing time and cost.

Although extraction time is not an absolutely important factor, it was included in this study as an independent variable based on previous studies. For instance, Wei *et al.* [16] demonstrated a significant correlation between time and bioactive compound yields (triterpenes).

This study stems from the significant potential and challenges associated with maximizing the industrial value of the Osaka tree. The results not only provide important and comprehensive scientific information on the bioactive profile of *Cassia fistula* L. fruit, but also provide support for the future development of high-value application products, e.g., safe and effective natural cosmetics and functional foods. Furthermore, the sustainable use of *C. fistula* fruit is consistent with the general goals of green and sustainable production.

STUDY OBJECTS AND METHODS

Materials. The pulp of *Cassia fistula* L. fruit was obtained from trees cultivated in Ho Chi Minh City, Vietnam. After harvesting fresh fruits, the seeds and husks were manually removed to isolate the fleshy pulp for extraction and analysis (Fig. 1).

Chemicals and equipment. The Folin-Ciocalteu reagent (Merck) was used to determine total phenolic content. The key equipment comprised a UV-Vis spectrophotometer (Shimadzu UV-1800), an infrared moisture analyzer (Ohaus MB45), and a blender (Fuki).

Phytochemical screening. Exactly 5 g of *C. fistula* fruit pulp was macerated in ethanol at a 1:30 (w/v) solvent-to-sample ratio for 8 h to obtain the primary extract. The extraction process was repeated twice under identical conditions. The three extracts were then combined, concentrated under reduced pressure, and subjected to a qualitative phytochemical analysis for bioactive compounds (Table 1) [17].

Determining the total phenolic content. The total phenolic content was determined according to the method developed by Sirichan *et al.* [19], with minor modifications to adapt to the experimental conditions. Briefly, 5 g of *C. fistula* fruit pulp was macerated in 30 mL of 98% ethanol in a 250-mL Erlenmeyer flask for 24 h at room temperature, followed by filtration to obtain crude extract. The extraction process (soaking and washing) was triplicated, and the combined extracts were pooled and adjusted to a final volume of 100 mL with ethanol.

For total phenolic content quantification, the extract was diluted 10-fold and analyzed using ultraviolet-visible spectroscopy at 765 nm, with gallic acid as the standard

a b

Figure 1 *Cassia fistula* L. tree (a) and *Cassia fistula* L. fruit (b)

Table 1 Phytochemical screening of bioactive compounds [18]

Qualitative compound	Methodology	Reaction result
Polyphenols and tannin	2 mL extract + 2 mL H_2O + 2–3 drops 5% $FeCl_3$	Dark blue, brown, blue
Alkaloids	2 mL extract + 3–4 drops Wagner's reagent	Red-brown precipitate
Flavonoids	2 mL extract + 2 mL 10% lead acetate	Yellow precipitate
Saponins	2 mL extract + 10 mL distilled water (boiled 2 min)	Persistent foam formation
Terpenoids and steroid	5 mL extract + 2 mL $CHCl_3$ + 3 mL concentrated H_2SO_4	Red-brown interface ring
Coumarins	2 mL extract + 3 mL 10% NaOH	Yellow coloration

reference compound. The total phenolic content, mg GAE/g DW, was calculated using the following formula:

$$\text{Total phenolic content} = C_x \times \frac{V_{dm}}{10^3} \times \frac{100}{a \times (100 - W)} \times K \quad (1)$$

where C_x is the measured gallic acid concentration, ppm, obtained from the standard curve; V_{dm} is the final volume of the standardized extract, mL; 10^3 served as the conversion factor from μg to mg; W is the sample moisture content, %; a is the initial sample mass, g; and K is the dilution factor applied when necessary.

Determining the moisture content. The moisture content was determined by the constant-weight drying method at 105°C [20].

Preliminary factor screening. Three key extraction parameters to be investigated included:

– extraction time: 5, 10, 15, 20, 25, and 30 min (fixed at 50°C and 1:30 g/mL solid-to-water ratio);
– temperature: 30, 40, 50, 60, and 70°C (fixed at 20 min and 1:30 g/mL ratio); and
– solid-to-water ratio: 1:10, 1:20, 1:30, 1:40, and 1:50 g/mL (fixed at 20 min and 50°C).

All experiments were triplicated, with the total phenolic content determined as previously described.

Optimizing the response surface methodology. Based on the preliminary screening results, we selected the optimal ranges for key extraction parameters for further optimization using the Box-Behnken experimental design: extraction time (15–25 min), temperature (40–60°C), and solid-to-water ratio (1:20–1:40 g/mL). A 15-run experimental matrix relied on the JMP 10 software (SAS Institute) to evaluate the effects of these parameters and their interactions on total phenolic content:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (2)$$

where Y is the total phenolic content; X_i is the independent variables (time, temperature, ratio); β_0, β_i, β_{ii}, β_{ij} denoted regression coefficients. The model adequacy was evaluated using R^2 and p-values.

Experimental validation. The optimal conditions predicted by the response surface methodology were experimentally validated in triplicate. To compare the predicted and the actual values, we used t-tests (Statgraphics).

Statistical analysis. After developing a multivariate regression model, we designed further experiments using the Box-Behnken approach implemented in the JMP 10 software (SAS Institute, USA). This statistical tool enabled optimization of extraction conditions for *C. fistula* fruit polyphenols while excluding variables that were not statistically significant ($p > 0.05$) based on experimental results.

To validate the model and assess the medicinal effects, we applied ANOVA followed by Tukey's honestly significant difference post-hoc test using the Statgraphics Centurion XI software (Statgraphics Technologies, Inc., USA). This analysis confirmed the model's reliability and revealed statistically significant differences ($p < 0.05$) between experimental treatments, ensuring the robustness of the research findings.

RESULTS AND DISCUSSION

Qualitative analysis of bioactive compounds in *Cassia fistula* L. fruit. Table 2 illustrates the preliminary qualitative results for secondary metabolite groups in *C. fistula* fruit. This analysis demonstrated a potential diversity in the chemical composition of the plant, with marked differences in the levels of presence of each metabolite group.

Three dominant groups of compounds were phenolic compounds, tannins, and flavonoids, which exhibited high contents (+++). *C. fistula* indeed proved to be a rich source of bioactive components with therapeutic potential. For phenolic compounds, their presence at highest levels correlated with antioxidant, anti-inflammatory, and antibacterial properties [21]. Tannins are complex polyphenols with properties similar to the phenolic group, which is prominent for its strong antioxidant and antibacterial profile [22]. Flavonoids also belong to the polyphenol group, known for its cardioprotective, anti-inflammatory, and antioxidant effects [23].

The list of compounds with moderate content (++) included coumarins, terpenoids, and steroids. Coumarins and their derivatives have clinically significant anticoagulant and anti-inflammatory effects [24]. Terpenoids possess reliable antibacterial and anticancer properties. Steroids include both hormonal compounds and phytosterols, which may contribute to the overall biological activity of the plant [25].

The compounds with low content (+) included alkaloids and saponins. Alkaloids possess potent pharmacological effects, but their presence is quite low, indicating

Table 2 Qualitative identification of secondary metabolites in *Cassia fistula* L. fruit

Compound class	Test method	Detection result	Relative abundance
Polyphenols	Folin-Ciocalteu	+++	High
Tannins	Ferric chloride	+++	High
Flavonoids	Aluminum chloride	+++	High
Coumarins	NaOH test	++	Moderate
Alkaloids	Mayer's reagent	+	Low
Terpenoids	Salkowski test	++	Moderate
Steroid	Liebermann-Burchard	++	Moderate
Saponins	Foam test	+	Low

that the therapeutic effect in *C. fistula* fruit is not high. Saponins were detected in trace form. They have characteristic foaming properties and biological activity, but their presence does not indicate any therapeutic efficiency [26].

Overall, *C. fistula* fruit pulp demonstrated a remarkable chemical composition with phenolics, tannins, and flavonoids. We conducted a detailed quantitative analysis to measure the concentration of each bioactive compound, thus creating a solid basis for the exploitation and application of the Osaka tree in medicine, cosmetics, and many other fields.

Effect of extraction time on total phenolic yield. According to Zainal *et al.* [27] and Zhang *et al.* [28], extraction time is an important factor affecting total phenolic content. Longer time often facilitates better solubilization and diffusion of phenolic compounds. Figure 2 shows that the total phenolic yield increased together with extraction time. Specifically, starting from the lowest value of 9.939 mg GAE/g DW recorded after 5 min, the total phenolic content showed a significant increase at subsequent times. After 10 min, the value rose to 10.273 mg GAE/g DW, followed by a sharp increase to 11.193 mg GAE/g DW after 15 min, reaching 11.587 mg GAE/g DW after 20 min.

However, after 20 min, the increase rate slowed down significantly, indicating a tendency towards saturation or equilibrium during the extraction process. When the extraction time reached 25 min, the total phenolic content only increased slightly to 11.598 mg GAE/g DW, showing an insignificant difference compared to the 20-min value. At the peak of the survey (30 min), the total phenolic content peaked at 11.699 mg GAE/g DW but showed no significant difference compared to the samples obtained at 20 and 25 min.

The total phenolic content tended to increase over time, but the pattern was not linear. Most of the rapidly soluble and diffusible phenolic compounds entered the solvent during the initial extraction stage [29]. As extraction time increased, the remaining phenolics were located deep within the cell structure or became more tightly bound. This phenomenon required either increasing the extraction kinetics or extending the time. In addition, the equilibrium established between the solid and liquid phases further slowed down the extraction process [30].

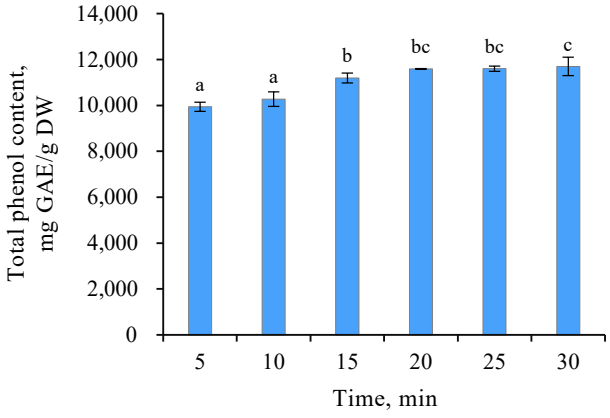
Thus, the extraction time had a significant effect on the phenolic recovery, especially in the early stages of the process. Extending the extraction time can help increase the total phenolic content, but not significantly. Therefore, determining the optimal extraction time is very important to balance the extraction efficiency and cost. In this study, 20 min was the best choice to recover most phenolics.

Effect of temperature on total phenolic yield. Figure 3 shows a complex correlation between the extraction temperature and the total phenolic content. In the range of 30–50°C, the phenolic extraction efficiency increased significantly, going from 9.27 to 11.02 mg GAE/g DW. This phenomenon could be explained by an increase in molecular kinetic energy and a decrease in solvent viscosity, which improved the diffusion and solubility of polyphenols into the extract. However, when the temperature exceeded 50°C (≤ 70°C), the total phenolic content dropped to 9.62 mg GAE/g DW, possibly due to the degradation of some thermosensitive polyphenols with increasing temperature. In addition, high temperatures might destroy their chemical structures or cause undesirable reactions [31].

Our results were similar to those reported by Vuong *et al.* [32], who examined the changes in the total phenolic content for *Carica papaya* L. In their case, the initial total phenolic content increased together with temperature, but as the temperature continued to increase, the total phenolic content decreased due to thermal decomposition.

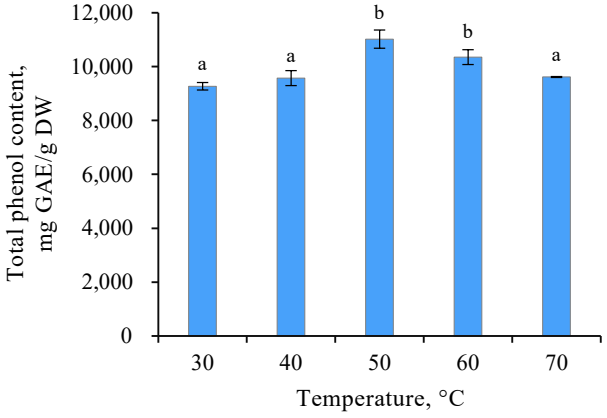
Based on experimental data, 50°C proved to be the optimal temperature to maximize the total phenolic yield from *C. fistula* This temperature balanced the enhancing mass transfer and the minimizing polyphenol degradation. For systematic optimization in further studies, the temperature range should be 40–60°C.

Effect of *C. fistula* fruit pulp/water ratio on total phenolic content. The raw material/solvent ratio is as



Different lowercase letters (a, b, c) indicate statistically significant differences at ($\rho < 0.05$)

Figure 2 Effect of time on total phenolic content



Different lowercase letters (a, b, c) indicate statistically significant differences at ($\rho < 0.05$)

Figure 3 Effect of temperature on total phenolic content

important for phenolic extraction as time or temperature. In [16], the optimal raw material/solvent ratio ranged from 1:10 to 1:50 (w/v), and the extraction process required heating.

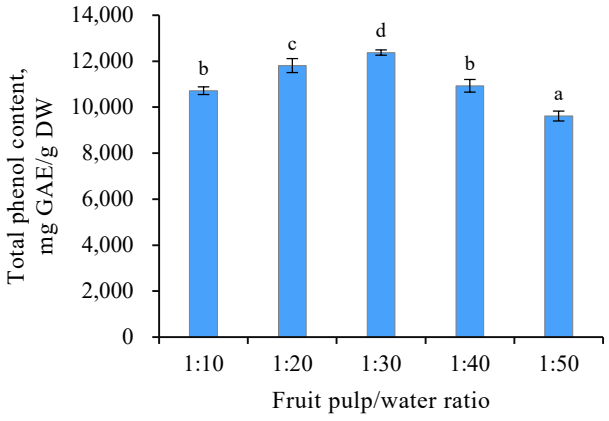
In this research, the raw material/solvent ratio between 1:10 and 1:30 (w/v) increased the total phenolic content significantly, from 10.72 mg GAE/g DW to a peak value of 12.38 mg GAE/g DW (Fig. 4). This phenomenon could be explained by the enhanced solubility and diffusion of phenolic compounds in larger solvent volumes [33]. A larger solvent ratio increased the contact surface area between the raw material and the solvent, thereby improving the mass transfer and maximizing the phenolic release into the extract [34].

If the ratio continued to increase beyond 1:30, the total phenolic content decreased significantly, dropping to 9.62 mg GAE/g DW at the ratio of 1:50. This decrease might be due to either the dilution effect of phenolic concentration [34] or the decreased solvent selectivity, meaning that when the solvent ratio went up, the viscosity of the solution went down, thereby reducing the solubility of phenolic compounds [35].

Sulaiman *et al*. [36] obtained similar results for phenolic extraction and reported the suitable solvent ratio as 1:40. Similarly, Myo & Khat-Udomkiri [37] observed that the solid/solvent ratio below 1:10 was not effective in extracting phenolic compounds, possibly because the solvent volume was not enough to dissolve the compounds.

The raw material/solvent ratio proved to be an important factor in achieving the highest phenolic yield. Based on the experimental data, we chose the ratio of 1:30 as the basis for the solvent ratio optimization experiment. The raw material/solvent ratio between 1:20 (lower limit) and 1:40 (upper limit) was selected to establish the optimal range.

Optimizing the extraction conditions. ***Optimal eximental modeling.*** In this study, we selected three optimization parameters, i.e., time, temperature, and solvent ratio. Table 3 presents the variation range for these factors.



Different lowercase letters (a, b, c) indicate statistically significant differences at ($\rho < 0.05$)

Figure 4 Effect of *Cassia fistula* L. fruit pulp/water ratio on total phenolic content

Table 3 Levels of experimental factors to optimize the extraction process

Independent variable	Symbol	Coded level		
		–1	0	1
Time	X_1	15	20	25
Temperature	X_2	40	50	60
Fruit/water ratio	X_3	20	30	40

Optimal extraction conditions. Table 4 shows the results of applying the JMP 17 pro-optimization software and the Box-Behnken model to the experimental matrix. The total polyphenol content fluctuated quite strongly, ranging from 6.30 to 11.37 mg GAE/g DW. This fluctuation shows the significant impact of various extraction parameters on the phenolic yield. The lowest total phenolic content (6.30 mg GAE/g DW) occurred under suboptimal conditions: short time (15 min), low temperature (40°C), and low solvent ratio (1:20 w/v). The highest total phenolic content (11.37 mg GAE/g DW) was achieved under a more favorable combination of time and solvent ratio.

We used a quadratic regression model to determine the correlation between the independent variables (X_1 – time; X_2 – temperature; X_3 – solvent ratio) and the total phenolic content objective function (Y):

$$Y = 10.56 + 0.53X_1 - 0.03X_2 - 0.42X_3 - 0.38X_1X_2 + \\ + 0.85X_1X_3 + 0.56X_2X_3 - 1.50X_1^2 - 0.70X_2^2 - 1.48X_3^2$$

Table 5 shows the optimal results, corresponding to the experimental data (Table 4) and combined with the Box-Behnken prediction model. The correlation coefficient of $R^2 = 0.9429$ was quite high, meaning that the regression equation explained > 94% of the actual data collected. In addition, the p-value of > 0.05 showed that the initial selection model was consistent with the experimental data. This result was also consistent with some previous studies. For instance, Szpisják-Gulyás *et al*. [14] reported an R^2-value of 0.94, which confirmed the accuracy and reliability of our approach.

The p-value of the regression equation was ≤ 0.05, indicating that the initially selected model was significant at 5%, i.e., the independent variables of time, temperature, and solvent ratio all had an effect on the final phenolic yield.

The correction coefficient $R^2_{adj} = 0.8402$ showed that the regression model explained > 84%, demonstrating a high accuracy of agreement between the experimental values and the predicted model values. In addition, Q^2 was a parameter to evaluate the model's ability to predict the accuracy. If $Q^2 > 0.5$ and R^2-$Q^2 < 0.3$, the model had a fairly high accuracy [38]. The Q^2 value in this case (0.8115) was considered satisfactory. Therefore, the initially selected model was able to predict the data generated by such factors as solvent ratio, extraction temperature, and extraction time.

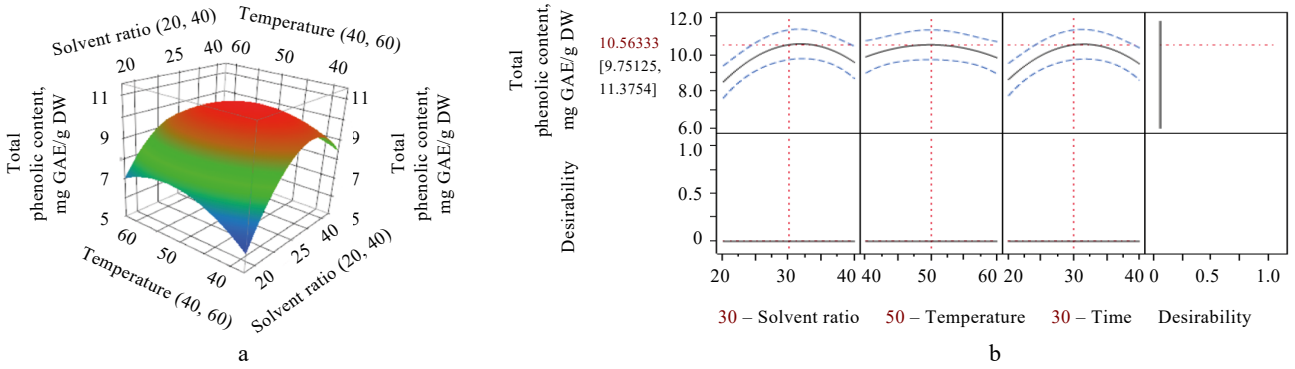
Table 4 Experimental design matrix and anticipated outcomes

Formula	Independent factor						Total phenolic content, mg GAE/g DW	
	X_1	X_2	X_3	Time, min	Temperature, °C	Fruit/water ratio, g/mL	Experimental value	Predicted value
1	0	+	+	20	60	40	9.63	9.54
2	–	0	–	15	50	30	7.67	7.35
3	0	0	0	20	50	30	10.04	10.24
4	+	0	–	25	50	30	7.11	7.25
5	0	0	0	20	50	30	10.28	10.24
6	–	+	0	15	60	40	8.17	8.14
7	+	+	0	25	60	40	8.39	8.27
8	0	–	+	20	40	20	8.45	8.48
9	–	–	0	15	40	20	7.58	7.46
10	0	+	–	20	60	40	7.21	7.25
11	0	–	–	20	40	20	8.26	8.38
12	0	0	0	20	50	30	10.37	10.24
13	–	0	+	15	50	30	6.30	6.35
14	+	0	+	25	50	30	9.20	9.26
15	+	–	0	25	50	20	9.32	9.38

"–" – low level encoding; "0" – center of variables; "+" – high level encoding

Table 5 Variance analysis and regression equation coefficients

Y	Coefficient	Standard error	p-value
Constant	10.563333	0.315916	< 0.001*
X_1	0.5375	0.193458	0.0390*
X_2	–0.02625	0.193458	0.8974
X_3	0.41625	0.193458	0.0841
X_1X_1	–0.38	0.273591	0.2235
X_2X_2	0.865	0.273591	0.0250*
X_3X_3	0.5575	0.273591	0.0972
X_1X_2	–1.507917	0.284763	0.0032*
X_1X_3	–0.690417	0.284763	0.0598
X_2X_3	–1.485417	0.284763	0.0034*
N = 20	R^2 = 0.9429	R^2_{adj} = 0.8402	Q^2 = 0.8115
Regression equation model: p-value = 0.0125*			
Lack of fit: p-value = 0.9617			



a b

Figure 5 Effects of solvent-to-material ratio, extraction temperature, and extraction time on total polyphenol content: (a) Response surface plots, (b) prediction profiler

The model yielded the following optimal extraction conditions: 21.15 min extraction time, 50°C extraction temperature, and 1:31.69 (w/v) solvent ratio. The optimal parameters given predicted the highest total phenolic content of 10.67 mg GAE/g DW (Fig. 5).

Experimental validation. The validation experiments were conducted with the predicted optimal conditions to evaluate the reliability and predictability of the optimization model. Table 6 compares the modeled total phenolic contents with those obtained by experimental extraction at the optimal conditions.

The difference between the predicted model and the experimental survey was very small (0.28%). The results demonstrated that the initially selected model was

Table 6 Total phenolic content obtained at optimal conditions

Factor	X_1, min	X_2, °C	X_3, g/mL	Total phenolic content, mg GAE/g DW
Predicted result	21.15	50	31.69	$10.67^a \pm 0.34$
Experimental result				$10.64^a \pm 0.03$

consistent with the experiment and demonstrated the high accuracy and predictive ability of the response surface methodology in determining the most effective extraction conditions for total phenolic yield from *C. fistula* fruit.

CONCLUSION

This study successfully optimized the extraction conditions for total phenolic yield from *Cassia fistula* L. (Osaka) fruit using the response surface methodology and the Box-Behnken model. The optimal results obtained from the initial selection model were as follows: the highest total phenolic content reached 10.64 mg GAE/g DW at 21.15 min extraction time, 50°C extraction temperature, and 31.69 (w/v) solvent ratio.

The optimized model was consistent with the experimental studies, providing reliable practical data for industrial scale-up of polyphenol extraction. These findings highlight the potential of *C. fistula* as an abundant natural source of such bioactive compounds as polyphenols, which can be used to develop new functional foods and pharmaceutical products.

CONTRIBUTION

D.V. Phuong and N.N. Thuan developed the research concept, designed the study, performed the experiments, collected the data, and drafted the manuscript. N.N. Thuan wrote the abstract and approved the final version.

CONFLICT OF INTEREST

The authors declared no conflict of interest regarding the publication of this article.

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AI DECLARATION

AI tools (ChatGPT (GPT-5), Grammarly (2025 version), Google Translate (2025 version), and QuillBot (2025 version)) were used to improve translation and increase readability, not to create content or change data. All manuscript content was carefully reviewed and edited by the authors after processing using AI tools.

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Additional information about the author

Do Viet Phuong ORCID 0000-0002-0081-0930
Nguyen Ngoc Thuan ORCID 0000-0002-1644-236X