Journal of Medicinal Materials, 2026, Vol. 31, No. 1+2 (pp. 96 - 104)

OPTIMIZATION OF THE EXTRACTION PROCESS FROM YELLOW CAMELLIA FLOWERS (*CAMELLIA CUCPHUONGENSIS* NINH & ROSMANN) COLLECTED IN PHU THO PROVINCE USING RESPONSE SURFACE METHODOLOGY

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Received February 25th, 2026 Accepted March 20th, 2026

Summary

This study was conducted to optimize the simultaneous extraction of total polyphenol content (TPC) and total flavonoid content (TFC) from the flowers of the yellow *Camellia* (*Camellia cucphuongensis* Ninh & Rosmann) collected in Phu Tho province. A Central Composite Design (CCD) combined with Response Surface Methodology (RSM) was employed to evaluate the effects of three independent variables: ethanol concentration (EtOH, 30-70%), extraction temperature (40-80°C), and extraction time (2-8 h). The optimal extraction conditions were identified as 58% EtOH, a temperature of 60°C, and an extraction time of 8 hours. Under these optimized parameters, the experimental values for TFC and TPC were 18.09 ± 0.17% and 35.12 ± 0.28%, respectively, which showed high compatibility with the predicted values. This optimized process provides a reliable scientific basis for the standardized production of bioactive-rich extracts from *C. cucphuongensis*. These findings highlight the potential of this endemic species as a high-value raw material for the development of antioxidant-based pharmaceuticals and functional foods in the field of natural products chemistry.

Keywords: *Camellia cucphuongensis*; *Optimization; CCD; Polyphenol; Flavonoid.*

1. Introduction

Yellow Camellias, commonly known as "Kim hoa Trà," refer to species within the genus *Camellia* L. characterized by yellow flowers in the Theaceae family, primarily distributed in Southern China and Vietnam. Globally, the *Camellia* genus comprises approximately 300 species, with 97 species found in China, of which 76 are endemic and about 30 produce yellow flowers [1]. According to recent studies, the *Camellia* genus in Vietnam consists of 95 species and 2 varieties, including 46 species specifically identified as yellow Camellias. Various research on yellow Camellias suggested that approximately 56 species have been discovered in Vietnam to date [2]. The chemical composition of yellow *Camellia* is similar to that of green tea (*C. sinensis*), containing numerous bioactive compounds such as phenolics, flavonoids, saponins, and essential oils [1].

Specifically, *Camellia cucphuongensis* Ninh & Rosmann (synonym of *Camellia flava* (Pitard) Sealy) is a notable yellow *Camellia* species primarily distributed in Ninh Binh and Phu Tho provinces [3]. Despite its potential, comprehensive research on this species remains relatively limited. Previous pharmacological investigations by Nguyen et al. have demonstrated the anti-diabetic and hypolipidemic effects of extracts derived from both the flowers and leaves of *C. cucphuongensis* [4],[5]. Furthermore, this research group reported significant antioxidant capacity along with high concentrations of TPC and TFC, particularly in the leaves [6]. The phytochemical profile of *C. cucphuongensis* has been significantly enriched by the findings of Do et al., who successfully isolated and identified four key phenolic markers from the flowers: kaempferol, gallic acid, astragalin, and vanillin [7]. These constituents are of profound pharmacological importance, as they are widely recognized for their potent antioxidant, anti-inflammatory, and neuroprotective properties [8]. The presence of these specific bioactive compounds not only corroborates the traditional medicinal use of the species but also provides a concrete scientific rationale for investigating targeted extraction processes to maximize their recovery for therapeutic applications.

While these findings establish a strong chemical and biological foundation, *C. cucphuongensis*, as an endemic species, remains insufficiently investigated regarding standardized extraction processes. Studies aimed at maximizing the simultaneous recovery of major

bioactive groups like TPC and TFC from the flowers are still scarce, which hinders the development of high-quality commercial products. In conventional extraction methods, parameters such as solvent concentration, temperature, and time are decisive factors [9]. To achieve optimal efficiency, it is essential to construct a mathematical model like Response Surface Methodology (RSM) to evaluate these interactions [10].

Therefore, this study was conducted to optimize the extraction process of TPC and TFC from the flowers of *C. cucphuongensis* collected in Phu Tho using a CCD model. The findings of this research will provide a scientific background for standardizing the preparation of bioactive-rich extracts, supporting the development of health products from this precious endemic medicinal resource.

2. Materials and Methods

2.1. Plant Materials

The flowers of *Camellia cucphuongensis* Ninh & Rosmann were harvested in October 2024 from the garden of BIOPHARM Hoa Binh Joint Stock Company (Hoa Binh city, Phu Tho Province). The species was authenticated by Prof. Dr. Tran The Bach. A voucher specimen (coded "Cong21102024") was deposited at the Institute of Biology, Vietnam Academy of Science and Technology. After harvesting, the flowers were washed and dried at 60°C until the moisture content was below 12%. The material was subsequently ground into a coarse powder (particle size < 2 mm) and stored in moisture-proof zip bags.

2.2. Chemicals and instrumentation

Standard gallic acid (98.7% purity, CAS: 149-91-7, Lot: CFS202502) and rutin (98.0% purity, CAS: 153-18-4, Lot: CFS202501) were purchased from Wuhan ChemFaces Biochemical Co., Ltd. (China). Folin & Ciocalteu′ s phenol reagent 2N was purchased from Merck (Germany). All chemicals and solvents used were of analytical grade.

The quantification was conducted on an AV-770 Double Beam UV-Visible/NIR Spectrophotometer (Jasco, Japan).

2.3. Extraction

Approximately 20.0 g of the plant material was accurately weighed and subjected to maceration with controlled heating at a solid-to-solvent ratio of 1:30 (w/v). This specific ratio was selected based on established studies identifying 1:30 as an optimal balance to maximize the concentration gradient for polyphenol and flavonoid recovery while ensuring solvent efficiency [11]. The extraction conditions, including ethanol (EtOH) concentration, temperature, and time, were established according to a CCD matrix. After the extraction process, the resulting mixture was filtered and concentrated under reduced pressure using a rotary evaporator to obtain the final extract. The TFC and TPC of the extracts were quantified using the methods described in Section 2.4.

2.4. Quantitative Analysis

2.4.1. Determination of TFC:

Principle: The TFC was determined using the aluminum chloride ($AlCl_3$) colorimetric assay, based on the formation of a stable chelate complex between flavonoids and Al^{3+} ions.

Preparation of standard curve: A series of rutin standard solutions (0.1, 0.2, 0.4, 0.6, and 0.8 mg/mL) was prepared from a 1 mg/mL stock solution.

Sample preparation: Approximately 0.1 g of the extract was accurately weighed and dissolved in 20 mL of 50% EtOH in a 25 mL volumetric flask, followed by ultrasonication. The solution was made up to volume and filtered.

Procedure: 1 mL of the sample or standard solution was transferred into a 10 mL volumetric flask. Sequentially, 0.3 mL of 5% $NaNO_2$ was added (5 min incubation), followed by 0.5 mL of 2% $AlCl_3$ (6 min incubation), and 0.5 mL of 1 M NaOH (10 min incubation). The flask was filled to the mark with distilled water. Absorbance was measured at 510 nm. TFC was calculated as rutin equivalents using the standard curve.

$$\text{TFC \%} = \frac{C_{tf} \times 25 \times P_f}{m_{tf} \times 10 \times (100 - B_f)}$$

C_{tf} (mg/mL) is the total flavonoid concentration (expressed as rutin equivalents) in the test solution, calculated from the standard curve equation; m_{tf} (g) is the mass of the test sample; P_f (%) is the purity of the rutin reference standard; and B_f (%) is the moisture content of the test sample.

2.4.2. Determination of TPC:

Principle: TPC was quantified by measuring the absorbance of the colored complex formed through the reaction with Folin–Ciocalteu (FC) reagent.

Preparation of standard curve: A series of gallic acid standard solutions (0.1, 0.2, 0.4, 0.6, and 0.8 mg/mL) was prepared from a 1 mg/mL stock solution.

Sample preparation: 0.1 g of the extract was extracted with 10 mL of 50% EtOH via ultrasonication for 30 minutes. The extraction was repeated, and the combined filtrates were made up to 25 mL in a volumetric flask.

Procedure: 100 μL of the sample or standard was added to 9.3 mL of 2% Na_2CO_3, followed by 0.5 mL of FC reagent. The mixture was incubated at 40°C for 1 hour. Absorbance was recorded at 760 nm. TPC was calculated as gallic acid equivalents.

$$TPC\ \% = \frac{C_{tp} \times 25 \times P_p}{m_{tp} \times 10 \times (100 - B_p)}$$

C_{tp} (mg/mL) is the total polyphenol concentration (calculated as gallic acid equivalents) in the test solution, derived from the standard curve equation; m_{tp} (g) is the mass of the test sample; Pp (%) is the purity of the gallic acid reference standard; and B_p (%) is the moisture content of the test sample.

2.5. Experimental Design and Optimization

The optimization process was conducted using a CCD implemented through Design-Expert 11.0 software. Three independent input factors were selected for investigation: ethanol concentration (EtOH), extraction temperature (ND), and extraction time (TG), which were coded in the experimental matrix as A, B, and C, respectively. The specific ranges for these factors are detailed in Table 1. The software generated a total of 20 experimental runs involving these three input variables. The dependent output variables (responses) were defined as TPC and TFC, which were evaluated according to the procedures described in Section 2.3.

Table 1. Experimental ranges and levels of the independent variables

Investigated Factor	Lowest (-α)	Highest (+α)	Low Level (-1)	High Level (+1)	Center Point (0)	Standard Deviation
EtOH (%)	16.4	83.6	-1 ↔ 30	+1 ↔ 70	50	16.95
ND (°C)	26.4	93.6	-1 ↔ 40	+1 ↔ 80	60	16.95
TG (h)	3.3	6.7	-1 ↔ 4	+1 ↔ 6	5	0.8516

The selection of independent variable ranges (Table 1) was based on preliminary single-factor experiments and established literature on the extraction of bioactive compounds from the *Camellia* genus [12]. Specifically, ethanol concentration (30–70%) was chosen to balance solvent polarity for diverse flavonoid and polyphenol recovery. Extraction temperature (40–80°C) was set to facilitate mass transfer without causing significant thermal degradation of thermo-sensitive compounds. These boundaries ensured that the experimental design space captured the true optimal regions while maintaining the stability of the target bioactive markers.

In this study, TPC and TFC were exclusively selected as the responses for the optimization model. While total extraction yield (% yield) provides information on the total mass recovery, it often includes significant amounts of non-bioactive ballast materials (such as sugars, fibers, and chlorophyll). For the development of high-quality medicinal extracts, prioritizing the concentration of specific bioactive markers is more critical for ensuring therapeutic efficacy than simply maximizing the total dry matter recovery [12].

2.6. Experimental Model Validation

To verify the compatibility between the theoretical results predicted by the CCD model and the actual experimental performance, the extraction process was re-executed under the determined optimal conditions. The experimental values for TFC and TPC were considered compatible with the model if the relative error did not exceed 5% compared to the software's predicted values.

2.7. Statistical Analysis and Data Processing

Design-Expert 11.0 software was utilized for the optimization of the extraction process input and output parameters. All experimental data were statistically processed using Microsoft Office Excel 2021.

3. Results and Discussion

3.1. Yields of Total Flavonoids and Total Polyphenols according to the CCD Model

Identifying critical input factors and their optimal values is essential for developing an effective extraction method. This study utilized a CCD to evaluate the influence of ethanol concentration (A), temperature (B), and extraction time (C) on the TFC and TPC.

Table 2 presents the TFC and TPC results for the 20 experimental runs. The TFC was calculated based on the linear relationship

between absorbance and rutin concentration, using the equation y = 1.0611x - 0.0108 with a correlation coefficient of R^2 = 0.9997. The TPC was determined via the linear regression equation between absorbance and gallic acid concentration: y = 0.6465x - 0.0137 with a correlation coefficient of R^2 = 0.9994.

Our results in Table 2 demonstrated the stability and reliability of the model through the center point replicates (Runs 15 to 20), where TFC and TPC values fluctuated within a narrow range of 14.92–15.49% and 33.07–33.61%, respectively, proving the reproducibility of the experiments.

Table 2. Experimental results for TFC and TPC

Std	Run	Factor 1 A:EtOH (%)	Factor 2 B:ND (°C)	Factor 3 C:TG (h)	Response 1 TFC (%)	Response 2 TPC (%)
1	1	30	40	4	11.72	28.59
2	11	70	40	4	12.71	29.68
3	4	30	80	4	12.71	27.43
4	7	70	80	4	14.02	27.89
5	6	30	40	6	12.45	28.03
6	15	70	40	6	14.14	28.82
7	12	30	80	6	12.61	31.28
8	3	70	80	6	14.46	30.43
9	10	16.4	60	5	11.62	26.47
10	5	83.6	60	5	13.65	27.95
11	19	50	26.4	5	11.63	27.9
12	9	50	93.6	5	11.54	26.93
13	2	50	60	3.3	14.5	32.15
14	13	50	60	6.7	16.76	34.38
15	14	50	60	5	14.92	33.49
16	16	50	60	5	15.01	33.07
17	20	50	60	5	15.49	33.1
18	18	50	60	5	15.19	33.07
19	17	50	60	5	15.19	33.61
20	8	50	60	5	15.05	33.31

The maximum levels of TFC and TPC were both obtained under the conditions of 50% EtOH, 60°C, and 6.7 hours (line 14), indicating a similar influence of extraction time on the yield of both TFC and TPC. Meanwhile, both EtOH concentration and temperature recorded a decrease in extractable active components compared to the regions surrounding the center point; this suggests the existence of quadratic parabolic effects and confirms that the optimal process region lies within the experimental design space. The consistent behavior of both TFC and TPC indices under the influence of independent variables indicated a high degree of uniformity in the diffusion and dissolution mechanisms of flavonoids and polyphenols in this solvent system, while also affirming that the harmonious coordination between solvent concentration and thermodynamic conditions is key to achieving maximum extraction efficiency.

3.2. Analysis of Variance (ANOVA) of the Two Output Variables

The ANOVA results presented in Table 3 show that the second-order quadratic regression models for both TFC and TPC response variables are highly statistically significant, with F-values of 42.80 and 97.04, respectively ($P < 0.0001$). For the TFC, the significant components include the linear effects of EtOH concentration (A) and extraction time (C), as well as the quadratic effects of EtOH concentration (A^2) and temperature (B^2) ($P < 0.05$). Based on these factors, the actual regression equation was established as follows: $TFC = -3.599 + 0.211A + 0.440B - 0.813C + 0.00015AB + 0.0077AC - 0.011BC - 0.0022A^2 - 0.0032B^2 + 0.157C^2$.

Regarding the TPC, in addition to the influence of EtOH concentration (A) and time (C), the interaction between temperature and time (BC) and the quadratic variables of EtOH concentration and temperature (A^2, B^2) exerted highly significant effect on TPC levels ($P < 0.0001$). The corresponding impact factors are expressed through the following equation: $TPC = 6.924 + 0.645A + 0.416B - 1.699C - 0.0007AB - 0.010AC + 0.049BC - 0.0054A^2 - 0.0052B^2 - 0.0091C^2$.

The "Lack of Fit" indices for both models were not statistically significant, with values of 0.0570 for TFC and 0.0591 for TPC (both $P > 0.05$), thereby confirming the suitability of the mathematical models with the obtained experimental data. Previous research indicated that an optimal model requires a non-significant "Lack of Fit" value to ensure that overlooked variables or random errors do not significantly impact the model's predictive capability [13].

Table 3. ANOVA results for the quadratic models of TFC and TPC

Source	F-value (TFC)	p-value (TFC)	F-value (TPC)	p-value (TPC)
Model	42.8	< 0.0001	97.04	< 0.0001
A- EtOH (%)	54.63	< 0.0001	7.51	0.0208
B - ND	4.41	0.062	0.0373	0.8506
C - TG	25.43	0.0005	36.1	0.0001
AB	0.2509	0.6273	4.17	0.0683
AC	1.67	0.2248	2.1	0.1779
BC	3.61	0.0867	49.42	< 0.0001
A^2	101.45	< 0.0001	431.84	< 0.0001
B^2	202.55	< 0.0001	403.22	< 0.0001
C^2	3.2	0.1041	0.0079	0.9307
Lack of Fit	4.71	0.0570	4.62	0.0591

The results presented in Table 4 further confirm the reliability of the model through the coefficient of determination (R^2), which reached very high values of 0.9747 and 0.9887 for the TFC and TPC models, respectively. This indicates that over 97% of the variation in active compound content can be explained by the input variables. Furthermore, the adjusted R^2 values for the TFC and TPC models were 0.9519 and 0.9785, respectively. The small discrepancy between the R^2 and adjusted R^2 values demonstrates that the variables included in the model significantly contribute to the response without causing overfitting.

Specifically, the high degree of compatibility between the adjusted R^2 and predicted R^2 values indicates that the model possesses strong predictive capability for the output variables. According to the study by Mourabet, a model is considered to have good predictive power if the difference between these two values is less than 0.2. The results of this study fully satisfied this criterion, with adjusted R^2 versus predicted R^2 values of 0.9519 vs. 0.8344 for TFC and 0.9785 vs. 0.9254 for TPC. This confirmed that the model not only fits the obtained data but also accurately predicts the outcomes of new experimental trials.

Table 4. Statistical fit parameters of the TFC and TPC regression models

Parameters	TFC	TPC
R^2 value	0.9747	0.9887
Adjusted R^2	0.9519	0.9785
Predicted R^2	0.8344	0.9254
Adeq Precision	21.5044	27.4551
Coefficient of Variation (C.V. %)	2.46	1.29

3.3. Interaction Effects Analysis

Analysis of the 2D contour plots in Fig. 3.1 reveals complex interactions between ethanol (EtOH) concentration (A), temperature (B), and time (C) affecting the extraction efficiency of active compounds from yellow *Camellia* flowers. The influence of the input variable pairs on TPC is illustrated in Figures 3.1 (1, 2, and 3), showing that the interaction between solvent concentration and extraction temperature plays a decisive role. This is evidenced by the concentric elliptical contour lines surrounding the optimal region.

The TFC reached its maximum level when the EtOH concentration was maintained within the 55–60% range in combination with a temperature of 60°C. According to the study by Savić et al. on the optimization of flavonoid extraction from tea, this combination optimizes solvent polarity. Simultaneously, the appropriate temperature helps weaken the hydrogen bonds between flavonoids and the cell matrix, thereby significantly enhancing the solubility of the active compounds in the extract [14]. Figures 1 (2 and 3) show steep

contour lines expanding toward the right corner of the plots, corresponding to the maximum extraction time of 6 hours. This confirms that time is a linear factor that helps the extraction system reach the highest concentration equilibrium.

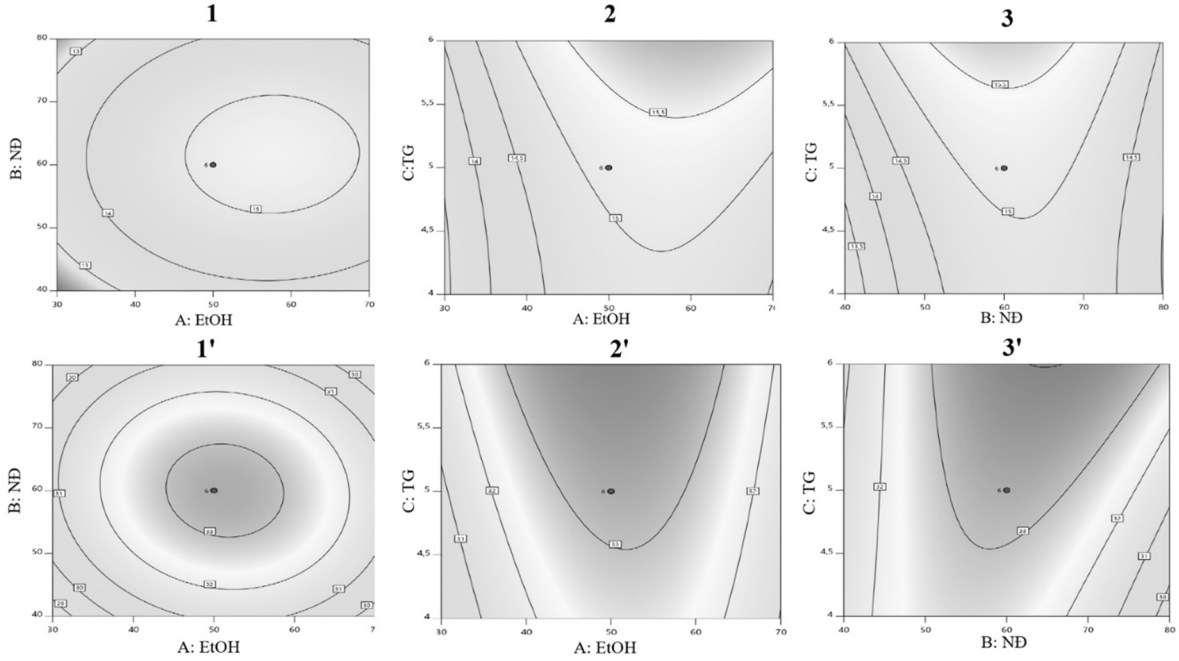

Fig. 1. 2D contour plots of the interaction effects on TFC (1, 2, 3) and TPC (1', 2', 3')

The influence of the input variable pairs on TPC, as shown in Figures 1', 2', and 3', exhibits a higher density of contour lines compared to TFC. This indicates that the polyphenol group is more sensitive to changes in the input conditions. Notably, Fig. 3' reveals that the interaction between temperature and time is statistically highly significant ($P < 0.0001$). The optimal region, indicated in dark red for concentrations exceeding 34%, converges clearly at the coordinates of 60°C and 6 hours. The increase in temperature reduces solvent viscosity, which, combined with the extended extraction time of 6 hours, allows the diffusion coefficient of phenolic compounds through the plant cell walls to reach its maximum. Research by Silva on the extraction of phenolic compounds suggested that the convergence of contour lines into a closed elliptical shape on a 2D plot serves as experimental evidence confirming the existence of an ideal interaction zone [15].

3.4. Determination of Optimal Conditions

Based on data from the CCD matrix, the study searched for optimal conditions within the established design space. The ethanol concentration (A) and extraction temperature (B) were maintained within the initial survey ranges of 30-70% and 40-80°C, respectively, as the research scope already covered the best extraction conditions. However, the extraction time (C) was extended to 8 hours, beyond the initial upper limit of 6 hours. This adjustment is supported by extraction kinetics in *Camellia* species, where specific flavonoids and polyphenols with bulky molecular structures require prolonged durations to achieve complete diffusion and solubility into the solvent system [16]. Furthermore, since the predicted optimal region for this variable was situated at the boundary of the initial design space, extending the range was necessary to prevent the exclusion of the actual global maximum [10]. This strategic refinement of the design space boundaries based on response surface trends ensures the mathematical model's robustness and enhances the reliability of the optimized predictions.

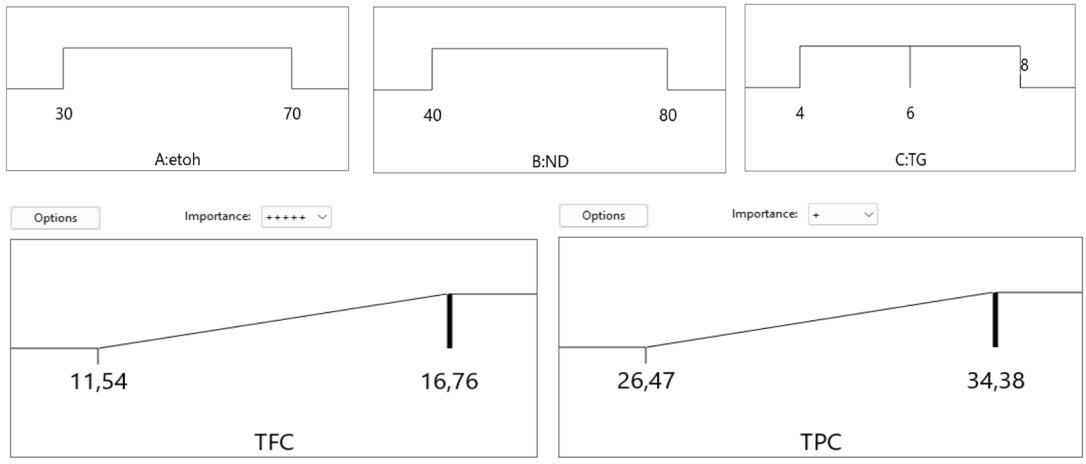

Fig. 2. Optimization criteria and desirability ramps for the extraction process

During the optimization setup for the two output variables, TFC was selected as the primary objective, while TPC was set as a supporting objective. The prioritization of TFC stems from the fact that flavonoids are a characteristic group of active compounds playing a key role in health protection effects, such as the potent antioxidant and anti-inflammatory activities of the yellow *Camellia* species [1],[2]. Assigning higher importance weights to compounds with more specific bioactivity ensures that the optimized extraction process targets the production of extracts with superior therapeutic efficacy. Fig. 2 illustrates the selection of these optimal conditions.

3.5. Results of CCD Optimization and Experimental Validation

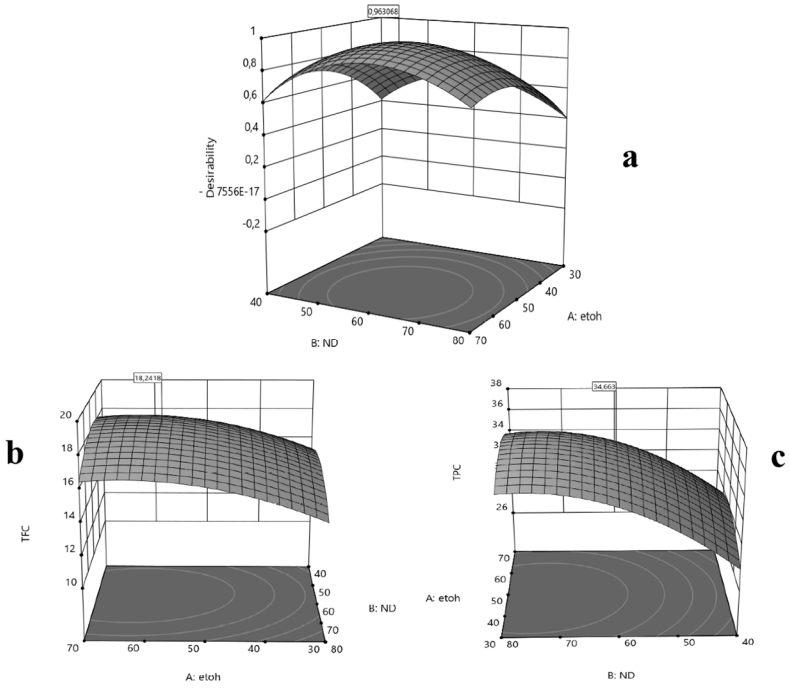

Fig. 3. Response surface and desirability plots for the optimization of TFC and TPC

The results from Design-Expert 11.0 software proposed 27 optimal solutions based on the established objective constraints, among which solution number 1 was selected due to its high

desirability index of 0.963. The optimal extraction conditions for yellow *Camellia* flowers, as determined by the software for the input variables, include an EtOH concentration of 57.664%, an extraction temperature of 59.983°C, and an extraction time of 8 hours. Under these parameters, the mathematical model predicts a TFC of 18.242% and a TPC of 34.664%.

Fig. 3 illustrates that increasing the EtOH concentration and temperature leads to a higher yield of active compounds. However, once these parameters exceed the optimal values, the active compound content tends to decrease slightly or reach a saturation point. Shiyan et al., in their research on the extraction of natural compounds from the *Camellia* genus, pointed out that this phenomenon occurs because changes in solvent polarity become unsuitable for dissolving the target solutes [16]. Furthermore, after reaching a certain threshold, the extraction process achieves a state of dynamic equilibrium, and efficiency no longer increases.

Additionally, the EtOH concentration was identified as the most significant factor affecting TFC ($P < 0.0001$) compared to temperature ($P = 0.0620$). This is consistent with the findings of Savić, who confirmed that the flavonoid group in tea was most efficiently extracted in the presence of EtOH at moderate concentrations [14]. Moreover, combining excessively high temperatures with high EtOH concentrations leads to a gradual decline in TFC. This is because high temperatures, while increasing molecular motion and disrupting plant tissues, simultaneously cause thermal decomposition or oxidation of heat labile compounds such as flavonoids and polyphenols [17].

Table 5. Comparison between predicted and experimental values under optimal conditions

Optimal Condition	Software Parameters (Predicted)			Actual Parameters (Experimental)		
	EtOH (A)	ND (B)	TG (C)	EtOH (A)	ND (B)	TG (C)
	57.664 %	59.983°C	8 h	58 %	60°C	8 h
TFC Content (%)	18.242			18.09 ± 0.17		
TPC Content (%)	34.664			35.12 ± 0.28		

To confirm the reliability of the model, validation experiments were performed in triplicate (n=3) under the optimal conditions, including an EtOH concentration of 58%, a temperature of 60°C, and an extraction time of 8 hours. The experimental results yielded an average TFC of 18.09 ± 0.17% and a TPC of 35.12 ± 0.28% (Table 5). The compatibility between the predicted and experimental values showed a relative error of less than 2%, which is well within the pre-established allowable limit of 5%. This high degree of similarity between the experimental data and the predicted data from the regression model is the most convincing evidence confirming the suitability of the second order quadratic model [18]. Furthermore, the optimized yields of TFC (18.09 ± 0.17%) and TPC (35.12 ± 0.28%) obtained for *C. cucphuongensis* in this study are remarkably high compared to other species in the *Camellia* genus, highlighting its superior potential as a source of bioactive compounds. For instance, a study on *Camellia dalatensis* leaves using response surface methodology reported an optimal TPC of 29.30%, which is notably lower than the value achieved in our current research [12]. This difference may be attributed to species-specific chemical profiles and the specific plant parts used, as flowers often accumulate higher concentrations of secondary metabolites than leaves. Compared to the well-known *Camellia sinensis*, which typically reports TPC ranges from 20% to 30%, the TPC and TFC levels of *C. cucphuongensis* remain highly competitive [19]. In another study for *Camellia nitidissima*, a widely used yellow tea species, TFC and TPC yields were reported at approximately 10-15% and 25-28%, respectively [20]. These comparisons demonstrate that *C. cucphuongensis* flowers are an exceptionally rich source of polyphenols and flavonoids, providing strong scientific evidence for the reliability of our optimized extraction process.

These validation results demonstrated that the CCD successfully established the parameters for input variables to optimize the two major bioactive groups from *C. cucphuongensis*, providing a stable extraction process with the potential for direct application in large-scale medicinal extract production.

4. Conclusions

Our study successfully applied a CCD to optimize the simultaneous extraction of TFC and TPC from the flowers of yellow *Camellia* (*Camellia cucphuongensis*) collected in Phu Tho. The optimal extraction condition was established as 58% EtOH, a temperature of 60°C, and a

duration of 8 hours, yielding a TFC of 18.09 ± 0.17% and a TPC of 35.12 ± 0.28%. The high degree of fit between the experimental results and the mathematical model predictions confirmed the accuracy and practical applicability of the process.

Furthermore, while this study successfully maximized the concentration of key bioactive markers, future research should consider incorporating total extraction yield (% yield) as an additional response variable. Investigating the correlation between mass recovery and bioactive potency will provide a more comprehensive evaluation of extraction efficiency, particularly for scaling up the process in industrial pharmaceutical production. These findings established a crucial foundation for the development of health products from this endemic yellow *Camellia*.

Acknowledgements: *The study was funded by Dai Nam University under project codes DT2425-22.*

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