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Optimization of Tannin Removal from Marigold Flowers by Water Extraction as Pretreatment for Lutein Recovery

Mukmin Sapto Pamungkas[a,b,c], Sherly Maharani Puspita Sari[a], Edia Rahayuningsih[a,b,c*]

[a]*Department of Chemical Engineering, Faculty of Engineering, Universitas Gadjah Mada, Jalan Grafika 2, Yogyakarta 55284, Indonesia*

[b]*Natural Dye and Extract Research Group, Faculty of Engineering, Universitas Gadjah Mada, Jl. Grafika No. 2, Yogyakarta 55281, Indonesia*

[c]*Indonesia Natural Dye Institute (INDI), Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia*

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ABSTRACT: Lutein extraction from marigold flowers (*Tagetes erecta* L.) with organic solvents is related to the presence of tannin-type phenolic compounds, capable of affecting the quality of food products. Therefore, this study aimed to evaluate the potential of water pretreatment to selectively extract tannins before lutein extraction. The experiment was conducted using water as a solvent for extraction due to its high and low selectivity for phenolic compounds and carotenoids, respectively. The extraction of tannins was investigated with respect to stirring speed, extraction time, and water-to-solid ratio using Response Surface Methodology (RSM) design of central composite. Tannins were extracted in a 1 L beaker at ambient temperature, and total phenolic content (TPC) was measured by spectrophotometry using the Folin-Ciocalteu method. The results showed that the quadratic model predicted the optimum extraction conditions as a stirring speed of 579 rpm, extraction time of 4.6 h, and water–solids ratio of 25 mL g^{-1} dry weight. At these extraction conditions, the process achieved a predicted TPC of 376.76 mg g^{-1} dry weight with 75.37% removal of tannin. Additionally, visible spectrophotometry confirmed the selectivity of the water-based extraction, as only 1.7% of the initial carotenoid content was present in the water extract.
Keywords: lutein; marigold; tannin; aqueous pretreatment; response surface methodology

1. Introduction

The growing demand for natural food colorants is intensifying the efforts to replace synthetic dyes with safer and plant-based alternatives (Novais et al., 2022). Despite these efforts, synthetic colorants like azo dyes are still widely used in food applications, accounting for approximately 65% of food dye additives (Barciela et al., 2023). Under reductive conditions, azo dyes can degrade into aromatic amines, which are recognized as potentially carcinogenic, raising serious food safety concerns (Balachandran & Sabumon, 2025; Ngo & Tischler, 2022). The issues have increased the interest in natural pigments that are visually appealing and biologically safer for human consumption (Masyita et al., 2025). Several plant-derived colorants have been explored as alternatives to synthetic dyes. These include curcumin from turmeric *(Curcuma longa)* as a substitute for tartrazine (Nandiyanto et al., 2017), carotenoids from pumpkin (*Cucurbita moschata*) peel (Perwitasari et al. 2023), betacyanins from red dragon fruit (*Hylocereus polyrhizus*) for red colorants (Pamungkas et al., 2020), chlorophyll from suji leaves *(Pleomele angustifolia Roxb.)* for green colorants (Rahayuningsih et al., 2018), and anthocyanins from butterfly pea flowers (*Clitoria ternatea L.*) for blue pigments (Syafa'atullah et al., 2020). The developments show the need for efficient and safe processing strategies to produce high-quality natural colorants suitable for food applications.

Among natural yellow food colorants, lutein, a xanthophyll carotenoid, has gained significant attention for the intense yellow–orange color and antioxidant properties (Zahara et al., 2024). Marigold (*Tagetes erecta L.*), a member of the Asteraceae family, is a major natural source of lutein, which is widely cultivated and distributed globally (Kusmiati et al., 2015). The characteristic yellow–orange coloration of marigold flowers is mainly due to carotenoid pigments, with lutein constituting approximately 80–90% of the total carotenoid content (TCC) (Abdel-Aal et al., 2015).

* Corresponding Author
Email address: edia_rahayu@ugm.ac.id

Dried marigold flowers typically contain 0.01–0.5% carotenoids, predominantly lutein and its esters, serving as a commercially attractive and sustainable source of lutein (Rahman et al., 2025). Due to the high lutein content, wide availability, and established use in food and feed applications, marigold flowers have become a major feedstock for the industrial production of natural yellow colorants, thereby reinforcing their relevance in the development of safer alternatives to synthetic dyes (Rodríguez-Amaya et al., 2023). Despite the advantages of marigold flowers as a natural source of lutein, the extraction process is associated with significant challenges. Conventional methods depend on organic solvents such as hexane, acetone, or ethanol, which co-extract tannin-related phenolic compounds along with carotenoids (Youssef et al., 2020). These tannins can impart undesirable astringency and interfere with mineral absorption, causing a reduction in the quality of lutein-rich extracts (Delimont et al., 2017). Consequently, their presence complicates downstream processing and limits the applicability of marigold-derived lutein. In previous studies, the removal of tannins as a pretreatment did not often include selective removal but rather saponification or solvent extraction (Fordos et al., 2025). Regarding physicochemical properties, lutein is non-polar and insoluble in water, while tannins are polar and soluble in water (Abda et al., 2025; Algan et al., 2020). This suggests that aqueous extraction can be used as a selective pretreatment. However, the method has not been comprehensively optimised, showing the need for effective strategies for tannin removal without loss of carotenoids.

For successful pretreatment with aqueous extraction to remove tannins, there is a need to understand the variables affecting the process. Generally, the extraction efficiency of tannins from plant materials is dependent on various operating variables, including stirring speed, extraction time, and solvent-to-solid ratio. Stirring increases mass transfer by improving turbulence, reducing the boundary layer thickness around the solid particles, and facilitating diffusion of the solute into the solvent (Wang & Mazzei, 2025). Extraction time affects the equilibrium between the solid and liquid phases, while the solvent-to-solid ratio influences the solubility of the solute and concentration gradients (Zhang et al., 2018). However, very high values of these variables can cause degradation, dilution, or under-exploitation of processing time, showing the need for process optimization instead of arbitrary choice. To overcome these challenges, Response Surface Methodology (RSM) offers a statistical method to assess the interactions among process variables and to optimize the operating conditions in a reduced number of trials (Montgomery, D.C., 2017). With the ability to model non-linear effects and variable interactions, RSM allows for the design of extraction processes, which facilitate the development of effective, scalable, and consistent pretreatment methods for natural product processing.

Previous studies have reported the selective removal of phenolic tannins from marigold flowers using aqueous extraction (water as a solvent) to reduce the carotenoid loss. The efficiency of tannin removal is dependent on the dominant process parameters, such as stirring speed, extraction time, and water–solids ratio. Therefore, this study aims to investigate and optimize selective aqueous extraction of tannin-related phenolics before lutein recovery as a pretreatment using RSM. The effects of these variables are evaluated with total phenolic content (TPC) and expressed as gallic acid equivalents (GAE), as the response variable. The results are expected to provide a strategy for tannin decontamination before lutein extraction, as well as enable the design of more selective and sustainable solutions for natural food colorants.

2. Materials and Methods

2.1. Materials

Flower bud samples of orange marigold (*Tagetes erecta* L.) were provided by Rumah Atsiri Indonesia (RAI) Karanganyar, Central Java, Indonesia, at 45 days after planting. Upon receipt, the flower petals were manually separated from the receptacles and used as the primary raw material for this study. The fresh petals were naturally dried under direct sunlight to achieve a constant weight. This process was conducted to reduce moisture content and prevent microbial degradation before extraction. Distilled water was obtained from the Separation Process Laboratory, Department of Chemical Engineering, Gadjah Mada University, Indonesia. Other material included ethanol (96%, Sigma-Aldrich), hydrochloric acid (37%, Merck), gallic acid (>98.5%, Sigma-Aldrich), Folin-Ciocalteu reagent (2N, Sigma-Aldrich), sodium carbonate (p.a., Merck), and β-carotene (98.5%, Sigma-Aldrich).

2.2. Determination of Moisture Content of Marigold Petals

The moisture content of the raw marigold petal materials was measured gravimetrically. Fresh marigold petals were weighed to determine the initial mass. The samples were dried in a laboratory oven at 100°C until a constant weight was reached, showing the complete removal of free moisture. After drying, the samples were cooled in a desiccator and reweighed. The moisture content was calculated as the difference between the initial and final masses, expressed as a percentage of the initial weight.

2.3. Determination of Tannin and Lutein Content in Marigold Flowers

Tannin and lutein were extracted from marigold flowers using an ethanol-based extraction method. Initially, 250 mL of ethanol was placed in a 500 mL three-neck round-bottom flask, and the pH was set to 5.1 using 0.1 N HCl. The extraction system was heated in a water bath until the solvent reached 65°C, as monitored with a thermometer inserted into the flask. After reaching the target temperature, 3 g of dried marigold petal powder was added to the flask, and the mixture was stirred at 150 rpm using a mechanical stirrer. The extraction was performed for 70 minutes at a constant temperature and with continuous agitation. After

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completion, the extract was filtered to separate the liquid extract from the solid flower residue.

2.4. Tannin Extraction Using Central Composite Design (CCD)

Tannin extraction from marigold petals was carried out in a 1 L glass beaker equipped with an overhead mechanical stirrer. The extraction system was operated with a fixed solid mass of 20 g, while the total working volume varied with the applied solid-to-water ratio, and agitation was provided by a two-blade impeller mounted on a vertical shaft. The overhead stirrer controlled the stirring speed, which ranged from 130 to 970 rpm. The extraction vessel was a beaker without baffles, and the experiments were performed at room temperature (25 ± 2°C). Subsequently, the dried petals were mixed with distilled water at solid-to-liquid ratios ranging from 9.8 to 35.1 mL g^{-1}, as specified in the experimental design (Table 1).

Table 1. Experimental design variables and levels for tannin extraction using CCD

Independent Variable	Symbol	Unit	−α	−1	0	+1	+α
Stirring speed	X_1	rpm	130	300	550	800	970
Extraction time	X_2	h	0.6	2.0	4.0	6.0	7.4
Solid-to-water ratio	X_3	ml/g	9.8	15.0	22.5	30.0	35.1

The extraction was conducted under the control of stirring speed (X_1), extraction time (X_2), and solid-to-water ratio (X_3). To systematically evaluate the effects of process variables on tannin extraction, RSM based on a CCD was used. Each variable was investigated at five coded levels (−α, −1, 0, +1, +α) to develop a second-order response surface model and evaluate linear, quadratic, and interaction effects. The ranges and coded levels of the independent variables used in the CCD are presented in Table 1. The experimental design consisted of 20 runs generated by the CCD matrix, including replicated center points to estimate experimental error. Each run was conducted under the conditions specified in the design, and the values obtained were used to build a response surface model and optimize the design. After extraction, the slurry was filtered. Subsequently, the concentrations of tannin and lutein in the extracts were determined using visible spectrophotometer, as response variables for the optimization analysis.

2.5. Determination of TCC (β-Carotene Equivalent (BCE))

The TCC of the marigold extracts was determined by visible spectrophotometric analysis, with results expressed as BCE. A β-carotene stock solution (100 ppm) was prepared by dissolving 1 mg of β-carotene in 10 mL of ethanol. The stock solution was diluted with ethanol to obtain a series of standard solutions at 0.5, 1, 2, 4, and 8 ppm. A calibration curve was prepared by plotting absorbance versus β-carotene concentration. For sample analysis, 0.2 mL of the sample extract was diluted to 10 mL with ethanol. Absorbance measurements of the standard and sample solutions were made at 425.6 nm. The analyses were carried out in triplicates and the average absorbance values were used. Equations (1) and (2) were used to determine BCE concentration and TCC, respectively:

$$\text{Sample BCE} = \frac{C_{carotenoid} \times V_{add} \times DF}{V_{sample}} \quad (1)$$

$$TCC = \frac{BCE \times V \times 10^{-3} \times 100}{W} \quad (2)$$

where BCE is β-Carotene Equivalent (μg mL^{-1}), TCC is the total carotene content (mg per 100 g^{-1} dry weight); $C_{carotenoid}$ is the carotenoid concentration obtained from the standard curve (ppm), V_{add} is the volume prior to spectrophotometric analysis (mL), DF is the dilution factor, V_{sample} is the volume of the analyzed sample (mL), V is the total sample volume (mL), and W is the sample mass (g).

2.6. Determination of TPC (GAE)

The amount of tannin-related phenolic compounds (TPC) was determined by the Folin-Ciocalteu method with the result expressed as the gallic acid equivalent (GAE) (Gong et al., 2012). Initially, a 100-ppm stock solution of gallic acid was prepared by dissolving 1 mg of gallic acid in 10 mL of distilled water. Acetone was used to dilute the stock solution to yield standard solutions of 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 ppm. A standard curve was generated using the absorbance of gallic acid. For the analysis, 0.5 mL of the extract or standard solution was combined with 2.5 mL of Folin–Ciocalteu reagent, which was diluted 10 times with distilled water (Pérez et al., 2023; Zugazua-Ganado et al., 2024). The solution was mixed properly and allowed to react for 5 min. Subsequently, 2.0 mL of a 7% (w/v) solution of sodium carbonate (Na_2CO_3) was added to the mixture, which was homogenized. The mixture was left at room temperature for 2 h for the reaction to take place. The absorbance was recorded at 754 nm by a visible spectrophotometer. Each sample was measured three times, and the average values were used to calculate the GAE concentration and TPC. Equations (3) and (4) were used to calculate the GAE and TPC, respectively:

$$GAE = \frac{C_{tanin} \times V_{add} \times DF}{V_{sample}} \quad (3)$$

$$TPC = \frac{GAE \times V \times D \times 10^{-3}}{W} \quad (4)$$

where GAE is the gallic acid equivalent (μg mL^{-1}), TPC is the total phenolic content (mg g^{-1} dry weight); C_{tanin} is the tannin concentration from the standard curve (ppm), V_{add} is the volume before the spectrophotometric measurement (mL), DF is the dilution factor, V_{sample} is the volume of the sample measured (mL), V represents the total sample

volume in milliliters (mL), and W denotes the sample mass in grams (g).

2.7. Calculation of Tannin Removal Efficiency

The percentage of tannin removal was calculated to determine the extraction efficiency of tannin removal from marigold flowers. This parameter represented the proportion of phenolic compounds removed from the solid matrix during the extraction process, providing a quantitative measure of performance under the applied operating conditions. The tannin removal percentage was calculated using Equation (5):

$$\%\, Tannin\ Removal\ = \left(\frac{TPC_0 - TPC_f}{TPC_0}\right) \times 100\% \qquad (5)$$

where TPC_0 is the initial TPC of the raw material (mg g^{-1} dry weight), and TPC_f is the TPC remaining after the extraction process (mg g^{-1} dry weight).

2.8. RSM and Analysis of Variance (ANOVA)

Optimization of tannin extraction process was carried out using RSM based on the CCD, as shown in Section 2.4. Data from 20 experimental runs were analyzed using Design-Expert® (version 13). Model adequacy and statistical significance were evaluated using ANOVA. The significance of the model terms was assessed based on the F-value and probability value (p-value), with a confidence level of 95% ($p < 0.05$) considered statistically significant.

3. Results and Discussion

3.1. Initial Tannin and Carotenoid Content of Marigold Flowers and Selective Extraction Behavior

The raw marigold petals used in this study had a moisture content of 31.67%, determined gravimetrically before extraction. This value is associated with freshly harvested marigold petals, which are considered during dry-weight-based calculations. Generally, ethanol extraction has been widely reported as an effective method for recovering carotenoids and phenolics from plant matrices (Rahman et al., 2025). The resulting values are used as reference points to evaluate extraction selectivity during the aqueous pretreatment.

As shown in Table 2, the initial TPC of the orange marigold flowers reached 499.85 mg GAE g^{-1} dry weight, while the TCC was 13,684.44 mg per 100 g dry weight. This high value showed that tannin-related phenolic compounds constituted a substantial fraction of the extractable components in marigold flowers. Previous studies reported considerably lower phenolic contents, typically 57.5–125.0 mg GAE g^{-1} DW when methanol maceration at room temperature for 24 h was used (Youssef et al., 2020). The higher TPC found in this study could be due to the use of a higher extraction temperature, agitation, and the greater polarity of ethanol compared with the methanol-water maceration systems used, which allowed the phenolic compounds to become soluble in the extraction solvent. Further, the marigold flowers were collected at 45 days after planting, a stage of flower that can produce higher levels of phenolic compounds.

The TCC in this study exhibited high carotenoid pigments in marigold petals. The reported values for carotenoids were 772 mg per 100 g dry weight in some marigold genotypes (Akshaya et al., 2017). However, the high content reported in this study was due to the flowering stage, flower genotype, drying process, and different extraction procedures adopted, which might affect the carotenoid contents. The carotenoid content was also measured as BCE, which was calculated spectrophotometrically. Additionally, marigold flowers are a good source of carotenoid pigments such as xanthophylls (lutein and zeaxanthin) that were extensively used as natural pigments (Deineka et al., 2008).

Table 2. Initial tannin and lutein content in fresh marigold flowers in ethanol solvent

Sample	Compound content in the sample	
	TPC (mg/g dry weight)	TCC (mg 100/g dry weight)
Orange marigold flowers	499.85	13,684.44

TCC was determined spectrophotometrically in the water extract to assess the selectivity of the aqueous extraction. Based on the analysis, TCC determined was 233.66 mg/100 g (dry weight), which corresponded to only 1.7% of the carotenoid content in the raw material (Table 3 run number 4). This showed that only a minor part of the lutein content was extracted into the water phase, while most of the carotenoids remained in the solid residues. To gain a better understanding of the compounds present in the aqueous extract phase, visible spectrophotometry was used.

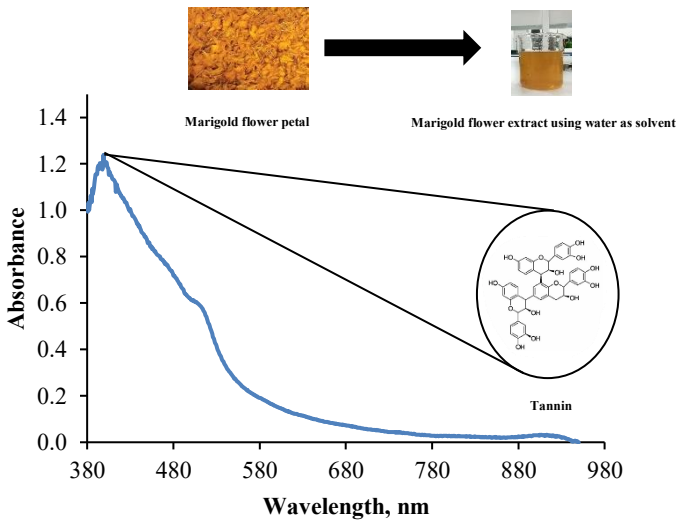

Figure 1. Visible absorption spectrum of marigold flower extract obtained using water as the extraction solvent

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As presented in Figure 1, the visible spectrum of the aqueous extract showed a major absorption band in the close UV region (390-400 nm), typical of phenolic compounds and tannin-containing extracts. These absorption bands were also observed for other tannins, tannic acid, and tannin-rich plant extracts, such as Angsana (*Pterocarpus indicus*) bark (Pamungkas et al., 2021), assigned to π–π* transitions of aromatic phenolic compounds. Meanwhile, carotenoids like lutein have a strong absorption in the range of 430-480 nm, attributed to conjugation of the polyene structure (Elkholy et al., 2023). These results showed that water selectively extracted tannin-related phenolic compounds without carotenoids.

3.2. Response Surface Modelling and ANOVA of Tannin Extraction

The experimental and predicted TPC values for each CCD run are presented in Table 3. According to the results, the experimental TPC values were in the range of 95.17-395.21 mg g^{-1} dry weight. This showed that the corresponding tannin removal efficiencies varied from 19.03% to 79.05%, indicating the potential of water as a solvent for the extraction of tannin-related phenolic compounds from marigold petals.

Table 3. Experimental versus predicted TPC values from the CCD-based tannin extraction model.

Std	Run	X_1	X_2	X_3	TPC actual, mg/g dry weight	TPC predicted, mg/g dry weight
4	1	300	2.0	15.0	208.86	142.31
14	2	800	2.0	15.0	230.38	176.40
5	3	300	6.0	15.0	228.34	216.71
20	4	800	6.0	15.0	232.25	235.29
19	5	300	2,0	30.0	199.63	193.65
17	6	800	2.0	30.0	221.54	230.23
9	7	300	6.0	30.0	215.92	266.96
3	8	800	6.0	30.0	224.43	288.03
13	9	550	4.0	22.5	365.60	366.74
1	10	550	4.0	22.5	389.72	366.74
16	11	550	4.0	22.5	370.71	366.74
8	12	550	4.0	22.5	395.21	366.74
6	13	550	4.0	22.5	332.04	366.74
15	14	550	4.0	22.5	347.87	366.74
18	15	970	4.0	22.5	250.79	236.68
7	16	130	4.0	22.5	172.02	190.30
10	17	550	7.4	22.5	339.44	274.97
12	18	550	0.6	22.5	95.17	163.81
2	19	550	4.0	35.1	363.57	292.37
11	20	550	4.0	9.8	129.50	204.86

where X_1 is the stirring speed (rpm), X_2 is the extraction time (hours), and X_3 is the water-solid ratio (mL g^{-1} dry weight).

The comparison of experimental and model-predicted TPC values showed that the model results fit well with the experimental data, based on the overall trend. This was particularly true for the center-point conditions (X_1 = 550 rpm, X_2 = 4 h, X_3 = 22.5 mL g^{-1}), where the TPC values of the repeated experiments were similar. The worst extraction efficiency was observed at X_2 = 0.6 (Run 18) with only 95.17 mg g^{-1} of phenolic compounds extracted, showing the need for a longer time for mass transfer to occur. Meanwhile, the highest TPC value of 395.21 mg g^{-1} was extracted under 550 rpm, 4 h, and a water-solids ratio of 22.5 mL g^{-1} (Run 12), with tannin removal efficiency of 79.05%. This shows that tannin removal efficiency is highly dependent on extraction conditions, suggesting the need for optimization of operating conditions.

A model suitability test was conducted to identify the most appropriate polynomial equation for fitting the CCD experimental data in Table 3. The results of the fit summary, which include sequential model p-values, lack-of-fit tests, and determination coefficients, are shown in Table 4. As presented in Table 4, the quadratic model showed a statistically significant sequential p-value ($p = 0.0052$). Meanwhile, the linear model ($p = 0.3679$) and the two-factor interaction model ($p = 0.9996$) were not significant ($p > 0.05$). The cubic model was not used due to insufficient degrees of freedom, leading to a lack of application for model development. The quadratic model had the highest adjusted R^2 (0.538) among the estimable models, showing greater explanatory power than the linear and 2FI models. Based on these statistical indicators, the quadratic model was selected to describe the relationship between stirring speed, extraction time, water–solids ratio, and the TPC response. The resulting quadratic regression equation is presented in Equation 6.

Table 4. Comparison of polynomial models for tannin extraction based on sequential p-values, lack-of-fit tests, and R^2 statistics.

Source	Sequential P-value	Lack of Fit P-value	Adjusted R^2	Description
Linear	0.3679	0.0022	0.0196	
2FI	0.9996	0.0011	-0.2055	
Quadratic	0.0052	0.0085	0.5380	Suggested
Cubic	0.0085		0.9278	Aliased

Table 5 shows the ANOVA results for the quadratic regression model describing the effect of stirring speed (X_1), extraction time (X_2), and water–solid ratio (X_3) on TPC. According to the results, the model explained 1.161×10^5 of the total 1.534×10^5 variability in TPC, showing the explanation of a considerable part by the regression model. The model was significant ($p = 0.0332$), showing that the variables used influenced the extraction response. Among the model terms, the quadratic effects (X_1^2, $p = 0.0072$; X_2^2, $p = 0.0089$, and X_3^2, $p = 0.0267$) were significant ($p < 0.05$) while the linear and interaction terms were insignificant. Therefore, the extraction response is driven by nonlinear effects, showing that optimal operating conditions for the studied variables exist.

Among the quadratic terms, X_1^2 (stirring speed) showed the largest sum of squares (42308.12) and the

smallest p-value. The results showed that stirring speed was the most influential variable for tannin extraction. This is explained by high mass transfer under agitation that increases solvent-solid contact, thereby facilitating diffusion of phenolic compounds into the solvent (Rahayuningsih et al., 2016; Eadmusik et al., 2022).

The adequacy of the quadratic model was further evaluated using diagnostic plots, as presented in Figure 2. The residuals versus predicted plot in Figure 2A showed that the externally studentized residuals were randomly distributed around zero without significant patterns, showing no significant outliers or systematic errors. Meanwhile, the predicted-versus-actual plot in Figure 2B showed reasonable correlation between experimental and predicted TPC values, with most data points close to the diagonal. The perturbation plot in Figure 2C showed that all variables had downward-opening parabolic trends, confirming the quadratic nature of the response. Among the studied variables, stirring speed showed the steepest curvature, indicating the highest sensitivity of TPC to this parameter according to ANOVA results.

$$\text{TPC (mg/g)} = 366{,}74 + 13{,}79X_1 + 33{,}05X_2 + 26{,}02X_3 - 3{,}88X_1X_2 + 0{,}6221X_1X_3 - 0{,}2712X_2X_3 - 54{,}18X_1^2 - 52{,}10X_2^2 - 41{,}76X_3^2 \quad (6)$$

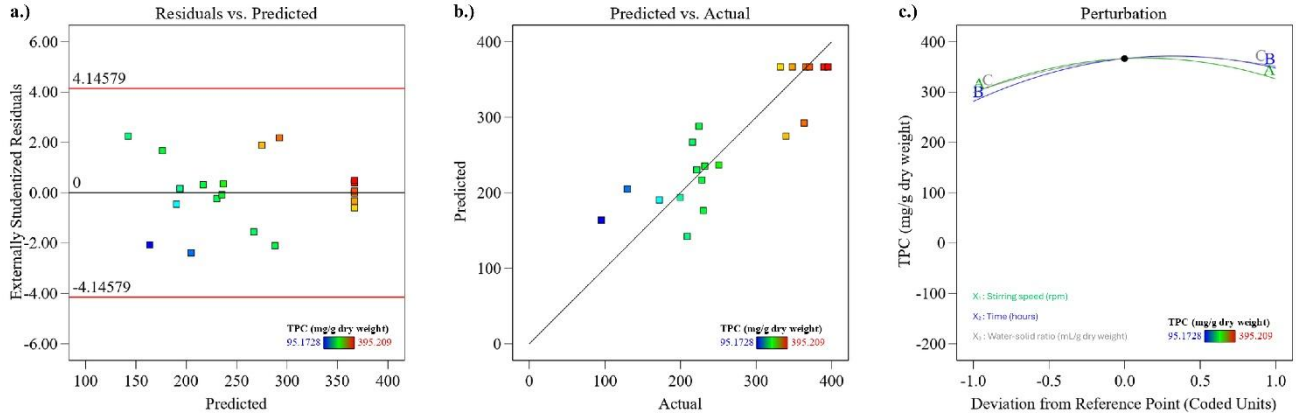

Figure 2. Model diagnostic plots include: a) Residuals versus Predicted values, b) Predicted versus Actual values, and c) Perturbation Curve.

3.3. Optimization of Tannin Extraction Conditions

Following the development of the quadratic regression model, RSM was applied to determine the optimal operating conditions for tannin extraction. The combined effects of stirring speed, extraction time, and water–solid ratio on TPC were shown by the three-dimensional response surface plots and contour plots presented in Figures 3–5.

Table 5. ANOVA results for the quadratic regression model of TPC during tannin extraction

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Coefficient Estimate	Standard Error
Model	1.161 x 10^5	9	12904.06	3.46	0.0332		
Intercept		1				366.74	24.91
X_1	2596.72	1	2596.72	0.6960	0.4236	13.79	16.53
X_2	14916.91	1	14916.91	4.00	0.0734	33.05	16.53
X_3	9245.86	1	9245.86	2.48	0.1465	26.02	16.53
X_1X_2	120.19	1	120.19	0.0322	0.8611	-3.88	21.60
X_1X_3	3.10	1	3.10	0.0008	0.9776	0.62	21.60
X_2X_3	0.59	1	0.59	0.0002	0.9902	-0.27	21.60
X_1^2	42308.12	1	42308.12	11.34	0.0072	-54.18	16.09
X_2^2	39112.18	1	39112.18	10.48	0.0089	-52.10	16.09
X_3^2	25135.87	1	25135.87	6.74	0.0267	-41.76	16.09
Residual	37309.15	10	3730.91				
Lack of Fit	34393.5	5	6878.70	11.8	0.0085		
Pure Error	2915.64	5	583.13				
Total	1.534 x 10^5	19					

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The response surface plots in Figures 3A, 4A, and 5A showed dome-shaped profiles, indicating an optimum region within the experimental domain. Consistently, the contour plots in Figures 3B, 4B, and 5B showed localized regions of maximum TPC, corresponding to the central operating ranges of the investigated variables. The trends are consistent with the significant quadratic effects identified in the ANOVA analysis (Table 5).

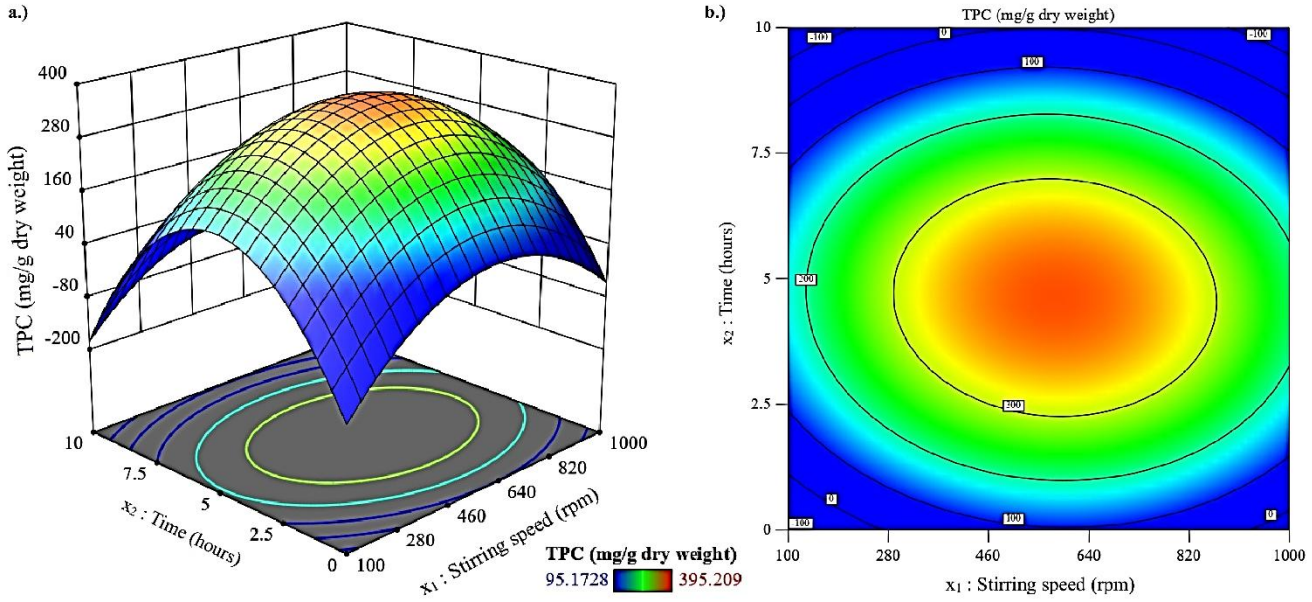

Figure 3. Three-dimensional response surface (a) and contour plot (b) showing the combined effects of stirring speed and extraction time on TPC (mg g^{-1} dry weight).

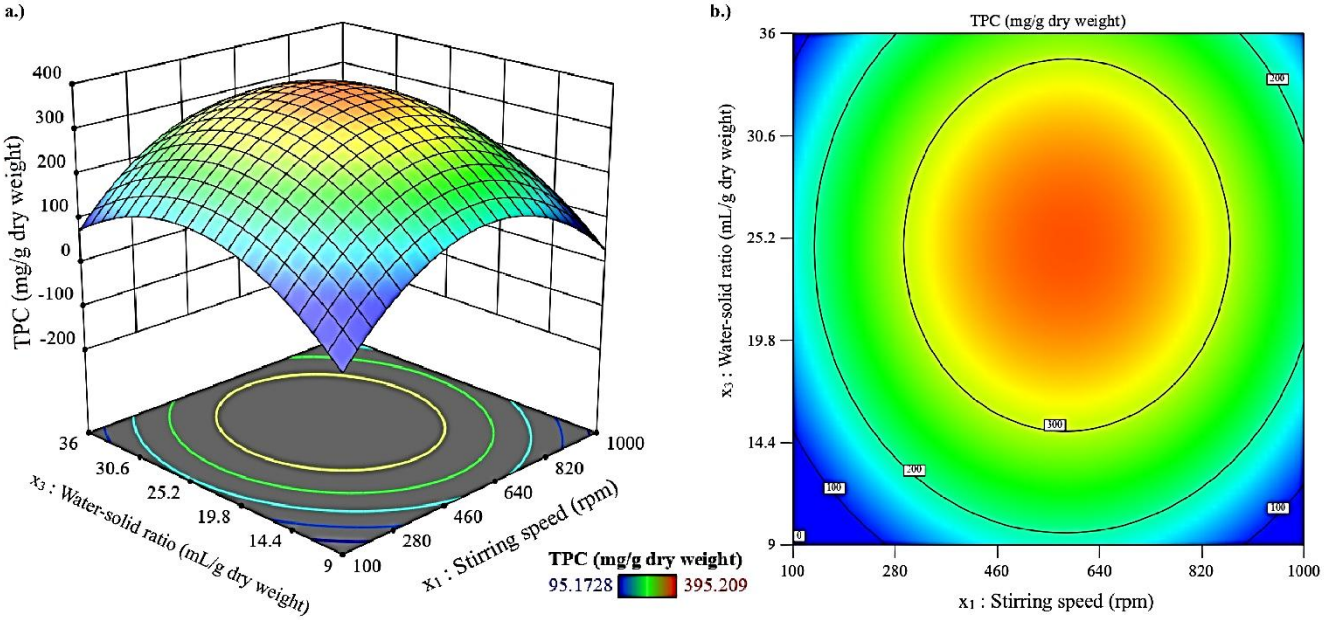

Figure 4. Three-dimensional response surface (A) and contour plot (B) showing the combined effects of stirring speed and water–solid ratio on TPC (mg g^{-1} dry weight).

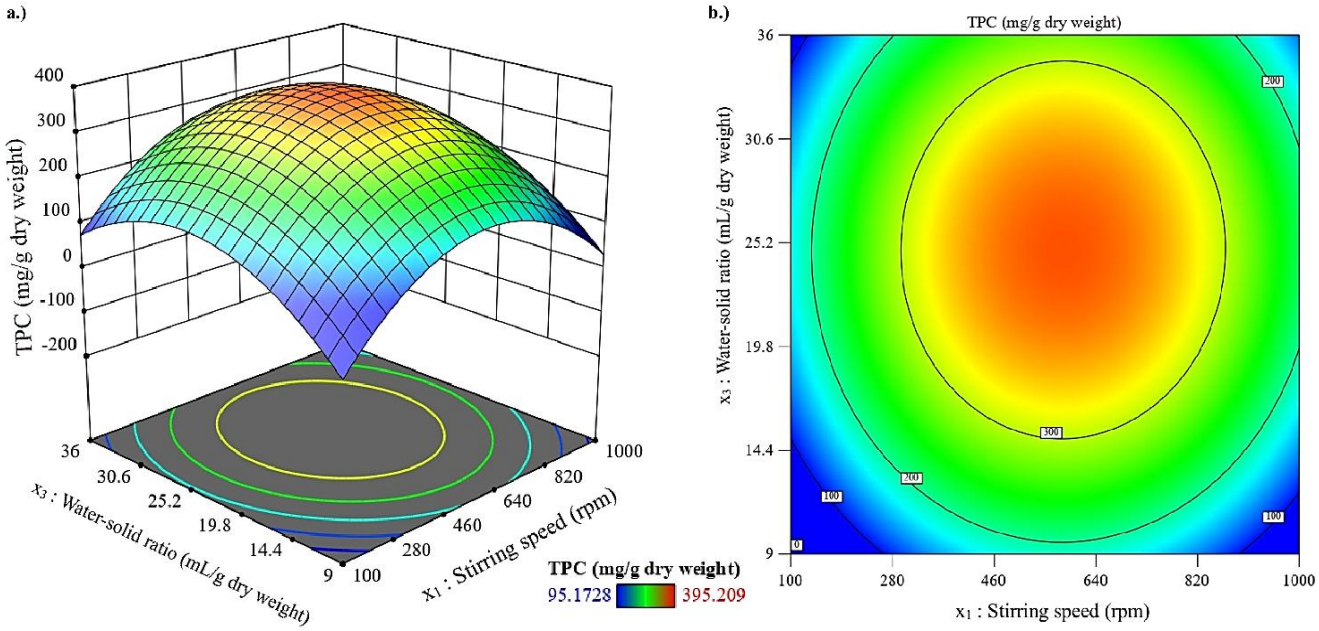

Figure 5. Three-dimensional response surface (A) and contour plot (B) showing the combined effects of time and water–solid ratio on TPC (mg g^{-1} dry weight).

This shows that the extraction process is primarily governed by nonlinear responses of the individual factors. Stirring speed had the most pronounced effect on the curvature of the response surface (Figures 3 and 4). Higher stirring speed was found to improve the tannin extraction efficiency as the mass transfer and solvent–solid contact were enhanced. However, further increase of the stirring speed had a negative effect on the tannin extraction. This effect was also observed with extraction time and water-to-solids ratio, with optimal values aiding tannin extraction. The longest extraction times and highest water dilution values significantly reduced TPC values (Figures 3-5).

The quadratic model was numerically optimized to provide optimal working conditions of a stirring speed of 579 rpm, an extraction time of 4.6 h, and a water-solids ratio of 25 mL g^{-1} dry weight. The predicted response TPC value was 376.76 mg g^{-1} dry weight, or 75.37% tannin removal.

4. Conclusions

In conclusion, this study shows the applicability of RSM based on CCD to model and optimize the aqueous extraction of tannins from marigold flowers. The quadratic model predicts the optimal extraction process at a stirring speed of 579 rpm, extraction time of 4.6 h, and a water–solid ratio of 25 mL g^{-1} dry weight. Under these conditions, the model estimates TPC of 376.76 mg g^{-1} dry weight, which corresponds to 75.37% tannin removal. The predicted response positively correlates with the experimental responses obtained under nearly center point conditions. Additionally, visible spectrophotometric analysis ensures the selectivity of the pretreatment solvent, as only 1.7% of the original carotenoid content is found in the aqueous phase. These results show that aqueous extraction can be a good strategy to reduce tannin without affecting carotenoids, thereby serving as a potential green pretreatment method for lutein recovery from marigold flowers.

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Statement

During the preparation of this study, the authors used ChatGPT (OpenAI) to enhance language and improve clarity in academic writing. After using this tool, the content was reviewed and edited as required, assuming full responsibility for the publication.

CRediT authorship contribution statement

Mukmin Sapto Pamungkas: Conceptualization, Methodology, Formal analysis, Validation, Supervision, Writing – review & editing; **Sherly Maharani Puspita Sari:** Investigation, Data curation, Formal analysis, Visualization, Writing – original draft; **Edia Rahayuningsih:** Conceptualization, resources, funding acquisition, project administration, supervision, writing – review & editing. All authors have reviewed and endorsed the final manuscript.

Declaration of competing interests

The authors declare that there are no competing financial interests or personal relationships.

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Data availability
The data may be shared upon request.

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