

RESEARCH ARTICLE

Optimisation of phytochemical compound extraction from *Cyperus rotundus* using central composite design

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Abstract - *Cyperus rotundus*, or purple nutsedge, is a plant native to Africa or India that has since spread globally, becoming a widespread and troublesome weed in tropical and warm-temperate regions, including Malaysia. Despite its negative impact on agriculture, this plant has been traditionally used for medicinal purposes and remains the focus of ongoing research for its potential pharmacological benefits. This study aims to evaluate and optimise the effects of extraction time (h) and agitation speed (rpm) on the yield of phytochemical compounds (total phenolic, total flavonoid, and antioxidant activity) from *C. rotundus* using response surface methodology (RSM) and central composite design (CCD). CCD was employed in Design-Expert software version 7.0.0.0 to optimise the extraction of phytochemical compounds from *C. rotundus*. The total phenolic compound (TPC), total flavonoid compound (TFC), and antioxidant activity (AA) were analysed using analysis of variance (ANOVA). The optimum condition is achieved at 5.12 h of extraction time and 195 rpm agitation speed, with TPC, TFC, and AA at their maximum values of 0.1399 mg/mL, 0.1778 mg/mL, and 0.8391 mg/mL, respectively. The results indicate that the interaction between extraction time and agitation speed had a significant effect on all phytochemical responses, whereas the individual effects of extraction time and agitation speed were significant only for AA extraction. The findings of this study are vital for conserving the therapeutic content of *Cyperus rotundus* during the processing stage and may enhance product quality in the future, which may aid the commercialisation of *Cyperus rotundus*.

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1. Introduction

Phytochemicals are naturally occurring biologically active chemical compounds found in plants that provide medicinal benefits to humans as therapeutic constituents and nutrients [1–2]. These compounds have biological properties such as antimicrobial action, antioxidant effects, immune system stimulation, modulation of detoxification enzymes, modulation of hormone metabolism, reduced platelet aggregation, and anticancer activity [3–4]. These compounds have biological activities such as antimicrobial activity, antioxidant activity, immune system stimulation, detoxification enzyme modulation, hormone metabolism modulation, reduction in platelet aggregation, and anticancer activity [3–4]. To date, researchers have identified over 4,500 phytochemicals and categorised them by therapeutic potential and physicochemical properties, with approximately 350 studied in detail [5–7]. Nutgrass (*Cyperus rotundus*) is more than just a weed. It is a highly invasive species, naturalised in tropical and subtropical regions worldwide, and it causes significant economic losses [8]. Crop losses caused by this species can range from 20% to 90% in both agronomic and horticultural systems worldwide. It is an ever-present and troublesome pest to farmers. However, its abundance creates possibilities. The extremely aggressive common weed, *C. rotundus*, destructive in agriculture, is also an easily available and affordable source of phytochemicals. An opportunity, in essence, to make a problem into a resource.

Many extraction techniques can recover valuable phytochemicals from this species. However, their efficiency depends on interactions among different factors. Extraction technique, temperature, particle size, extraction time, solvent type, matrix porosity, solvent concentration, pH, sample-to-solvent ratio, and solvent diffusivity affect the results. [9–10]. These variables affect overall phenolic yield and antioxidant properties, which are vital for the plant's medical and industrial significance [10]. Despite this potential, optimisation of extraction parameters for *C. rotundus* remains limited. Existing literature is largely limited to individual parameters or outcomes, e.g., total phenolic content or antioxidant activity. For instance, [11] optimised phenolic extraction and evaluated antioxidant activity, while [2, 12] studied the bioactive properties of antioxidants and the anti-inflammatory properties of tube extracts. However, these studies focus mostly on individual parameters or specific extraction aspects and do not comprehensively address multiple phytochemical outputs. The interaction effects of process variables, such as extraction time and agitation speed, have not been explored, even though these interactions could affect overall efficiency.

Systematic optimisation using response surface methodology (RSM) to optimise multiple phytochemical outputs has not been well explored. The application of RSM in *C. rotundus* remains in its early stages. The use of RSM for *C. rotundus* remains limited. Previous work used basic tools and software [13]. For instance, data analysis is performed using Microsoft Office Excel, an approach that is not suitable for analysing interaction effects or for sound process optimisation. This issue has been well documented recently because simplified methods not only require more experiments but also fail to capture synergistic variable effects, while RSM provides a statistical model designed to model interactions and identify optima [3, 10]. Recent studies in natural products research have shown that central composite and Box–Behnken designs outperform conventional designs in prediction accuracy and extraction efficiency [3, 10]. RSM is a technique that designs

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experiments, models interactions, evaluates variable effects, and determines optimum conditions with fewer experiments [14–15]. The central composite design (CCD) is the advisable model for process optimisation. Hence, this work aims to study and optimise extraction time and agitation speed for three key outputs: antioxidant activity (AA), total phenolics content (TPC), and total flavonoids content (TFC). The aim is to find the best extraction conditions to obtain the highest yield of these compounds with the least experimental time and cost.

2. Materials and Methods

2.1 Materials

C. rotundus was collected from nearby locations at University Malaysia Pahang Al-Sultan Abdullah (UMPSA) in Gambang, Pahang, Malaysia. After cleaning and rinsing with water to remove dust, the freshly collected *C. rotundus* was oven-dried for 72 h at 40 °C. All dried materials were ground into a powder using a grinder and sieved for fine particles before being sealed in a container [16].

2.2 Experimental Design

The extraction of phytochemical compounds from *C. rotundus* was optimised using a CCD with two independent variables in Design Expert software 7.0.0. This study optimised extraction time (h) and agitation speed (rpm) as the two most important variables. We selected these ranges from literature-reported values to make them relevant and applicable to *C. rotundus* extraction. The selected ranges were based on the study of [17], which investigated the effect of extraction parameters on flavonoid content and antioxidant activity. Table 1 presents the selected factors and ranges used to optimise phytochemical extraction conditions. TPC (mg/mL), TFC (mg/mL) and AA (mg/mL) were measured as the dependent variables. We carried out 13 experimental runs under randomised conditions, as shown in Table 2, with five centre points (200 rpm agitation speed and 5 h extraction time). The experiment sequence was randomised to reduce bias, and each experiment was repeated three times to ensure the validity of the statistical analysis.

Table 1. Selected variables and ranges

Factors	Range
A – Extraction time	4 – 6 h
B – Agitation speed	160 – 240 rpm

2.3 Preparation of Extract for Phytochemical Screening

The extraction conditions were carried out under randomised conditions developed by Design Expert software (version 7.0.0). First, 5 g of powdered *C. rotundus* sample was added to 85% methanol and stirred at room temperature. The mixture was transferred to an incubator shaker set at 80 °C, and the extraction time and agitation speed were set according to the conditions in the Design Expert table. The sample was extracted, then centrifuged at 4000 rpm for 20 min at 25 °C. The extracts obtained were filtered through Whatman filter paper and kept at 4 °C for further analysis. To ensure reliable results, the methanol concentration (85%) and temperature (80 °C) were kept constant throughout the process [18].

2.4 Phytochemical Screening

2.4.1 Determination of TPC

The Folin-Ciocalteu method was used to measure TPC as described by [19]. In brief, 0.5 mL of the sample was mixed with 2.5 mL of Folin–Ciocalteu reagent and allowed to react for 5 min. Then, 2 mL of sodium carbonate was added, and the mixture was incubated in the dark for 1 h, after which its absorbance was measured at 750 nm using a spectrophotometer. Gallic acid was used to generate the calibration curve for quantification.

2.4.2 Determination of TFC

The TFC was measured by the Dowd method as described by [20]. In summary, 1 mL of sample was mixed with 0.3 mL of 10% aluminium trichloride solution and left to stand for 5 min. Then, 2 mL of 1 M sodium hydroxide was added, allowed to rest for 6 min, and 4.4 mL of distilled water was added. The absorbance of the final mixture was measured at 450 nm in a spectrophotometer. Quercetin was used as the standard for the calibration curve and was quantified.

2.4.3 Determination of antioxidant activity

The AA was evaluated by a modified DPPH free radical scavenging assay according to the method of [21]. The DPPH assay is a simple, widely used technique to assess antioxidant activity based on a compound's ability to scavenge free radicals [22]. About 1 mL of the sample was added to 2 mL of 0.1 M DPPH solution in methanol and incubated at room temperature in the dark for 30 min. The absorbance of the mixture was then determined at 366 nm on a spectrophotometer, and quercetin was used as the standard to prepare a calibration curve.

2.5 Data Analysis Using Design Expert

All data were recorded in Design Expert software 7.0.0. The responses were analysed to determine the optimum extraction conditions and the interaction between the factors.

2.6 Validation Experiment

The best conditions suggested by Design Expert software 7.0.0 were further validated to verify the model. The percentage error was then calculated to evaluate prediction accuracy.

3. Results and Discussion

3.1 Experimental Data

Table 2 shows the 13 experimental runs and the phytochemical analysis results for TPC, TFC, and AA. The responses were analysed statistically using analysis of variance (ANOVA) at a 95% confidence level to ensure the reliability of the results. The TPC values ranged from 0.1314 mg/mL to 0.1401 mg/mL, and the TFC values ranged from 0.0238 mg/mL to 0.2005 mg/mL, as shown in Table 2. As shown in Table 2, the AA ranged from 0.8230 mg/mL to 0.8423 mg/mL. These differences reflect how experimental conditions affect extraction efficiency. The results were statistically significant, as shown by ANOVA, which showed that the extraction parameters influenced the yields of bioactive compounds.

Table 2. Experimental results of TPC, TFC, and AA

Standard Order	Factor 1: Extraction time (h)	Factor 2: Agitation speed (rpm)	Response 1: TPC (mg/mL)	Response 2: TFC (mg/mL)	Response 3: AA (mg/mL)
1	4.50	180	0.1314	0.0510	0.8247
2	5.50	180	0.1377	0.0909	0.8367
3	4.50	220	0.1371	0.0994	0.8325
4	5.50	220	0.1319	0.0238	0.8275
5	4.00	200	0.1334	0.0468	0.8230
6	6.00	200	0.1338	0.0697	0.8296
7	5.00	160	0.1355	0.0434	0.8307
8	5.00	240	0.1334	0.0279	0.8247
9	5.00	200	0.1399	0.1960	0.8412
10	5.00	200	0.1401	0.1887	0.8423
11	5.00	200	0.1401	0.1985	0.8396
12	5.00	200	0.1399	0.2005	0.8402
13	5.00	200	0.1399	0.1887	0.8419

3.2 Data Analysis of TPC on Design Expert

Analysis of variance (ANOVA) was used to examine the model's coefficients, the effect of individual variables, and variable interactions to estimate TPC (Table 3). The "Prob > F" value is an important piece of information in the ANOVA output and indicates the model's statistical significance. The "Prob > F" value is 0.0052, indicating that the model terms are significant and that the selected factors and interactions had a measurable effect on TPC in this case. That is, the interaction of factors A and B (AB), the square of factor A (A^2), and the square of factor B (B^2) were significant contributors to the accuracy of the model, as indicated by a p-value less than 0.05. However, factors A and B were unimportant, based on their high p-values. The F value for the TPC model was 9.40, which means that the model is statistically significant and the probability that the result is due to noise was only 0.52%. Extraction time and agitation speed had no individual significant effect on TPC; however, the interaction between these two parameters suggests that phytochemical extraction from *C. rotundus* depends on their combined effects. Agitation improves contact between the solvent and the solids and reduces the boundary layer around the plant particles, and the extraction time is adequate for diffusion and movement toward equilibrium [23]. Thus, the synergistic effect of adequate time and proper agitation results in maximum mass-transfer efficiency and, consequently, maximum recovery of phenolics, flavonoids, and antioxidant activity.

Furthermore, the model's R^2 is 0.8704, indicating a good fit. A model with an $R^2 \geq 0.80$ is typically regarded as acceptable for bioprocess models [24]. The R^2 value of this model is 0.8704, indicating good predictive ability and that the model can represent the TPC extraction process well. The high R^2 value indicates that the model fits the experimental data well and can be used to gain insights into the behaviour of the phytochemical extraction process under various conditions. This good model fit gave them confidence in their experimental design and provided a structure to follow when planning further optimisation with minimal trial and error.

Table 3. ANOVA table for TPC

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.193E-004	5	2.385E-005	9.40	0.0052	Significant
A: Extraction time	2.760E-007	1	2.760E-007	0.11	0.7512	
B: Agitation speed	1.529E-006	1	1.529E-006	0.60	0.4630	
AB	3.275E-005	1	3.275E-005	12.91	0.0088	
A ²	6.213E-005	1	6.213E-005	24.49	0.0017	
B ²	4.615E-005	1	4.615E-005	18.19	0.0037	
R ²	0.8704					
Adj. R ²	0.7778					

The response surface plot showing the interaction between extraction time and agitation speed on TPC was developed from the quadratic model. Figure 1 (a) presents the three-dimensional interaction of TPC, extraction time, and agitation speed. Individual one-factor plots of Figure 1(b) and (c) give a more detailed picture of the functions of TPC with respect to extraction time and agitation speed, respectively. In Figure 1(b), TPC first rises as extraction time increases up to 5 h and then drops. This indicates that extraction times longer than 5 h are unnecessary, as they increase the likelihood of oxidation of phytochemical compounds in the extract. In long-time extractions, phenolic compounds may degrade by oxidation, resulting in a decrease in TPC. Similarly, the data in Figure 1(c) indicate that TPC increases with increasing agitation speed until it reaches 200 rpm, then decreases. The reduction in TPC with increasing agitation speed is probably caused by the mechanical breakdown of phenolic compounds in the extract [6]. The TPC behaviour observed in the present study agrees well with reported results in recent extraction studies. For instance, [25] reported that the phenolic content in *Croton Macrostachyus* had reached the optimum level at the optimum extraction time and decreased with the increase in the extraction time, due to the degradation of the phenolic compounds during longer extraction time.

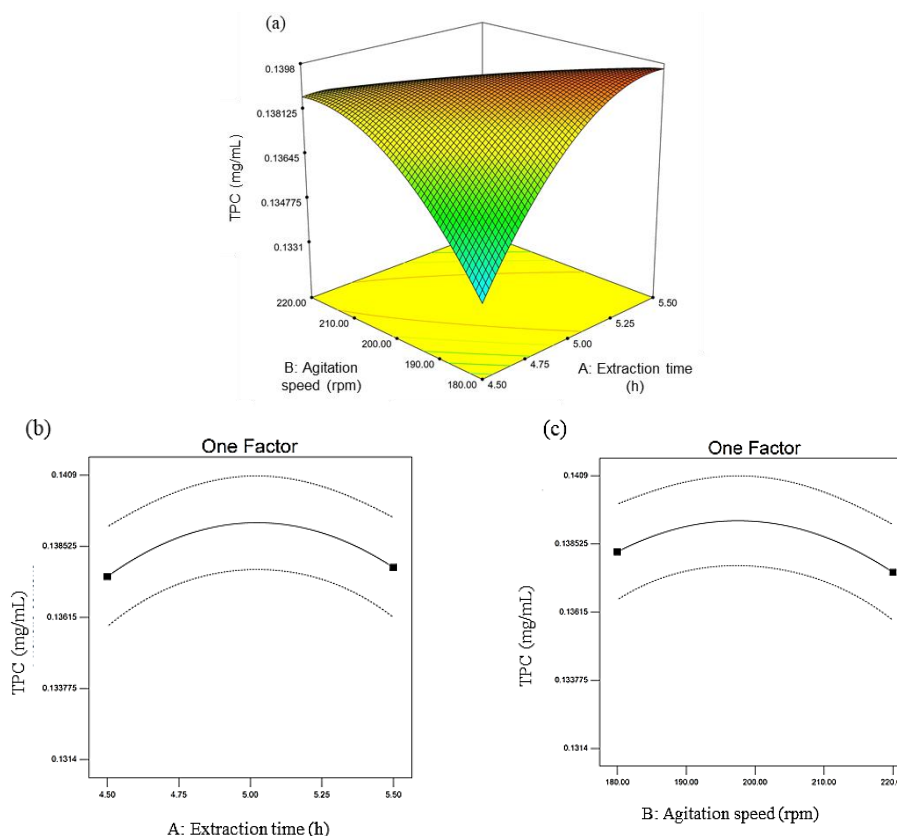


Figure 1. (a) 3D response surface on TPC as a function of extraction time and agitation speed, (b) effect of extraction time on the TPC, and (c) effect of agitation speed on the TPC

Similarly, extraction time and technique significantly affected phenolic yield in spice extracts, as shown by the optimum microwave-assisted extraction of 1.263 mg GAE/g DW reported by [25], which revealed that longer extraction times did not necessarily yield more phenols. In terms of agitation speed, the results demonstrated that TPC increased with agitation speed up to an optimum value of 195 rpm and then decreased, which is in agreement with the results of

[8], who reported that the optimum agitation speed for *Asystasia Gangetica* was 204 rpm with a corresponding TPC value of 0.2086 mg/mL. This study also reported that agitation rates above the optimum resulted in lower phenolic yields because of mechanical or oxidative degradation. If the agitation speed is too high, the shear force will be too great, which will result in a decrease in the concentration of the phenolic compounds. Therefore, extraction time and agitation speed should be optimised to maximise TPC and minimise compound degradation.

3.3 Data Analysis of TFC on Design Expert

Table 4 presents the ANOVA results for TFC, which were used to assess the model coefficients, variables, and potential interactions. The "Prob>F" value of 0.0065 suggests that the model terms are statistically significant. Specifically, the quadratic terms for the two variables, A^2 and B^2 , are significant contributors to the model, as shown by p-values below 0.05. Meanwhile, factors A and B individually showed no significant effect on the process, as evidenced by their high p-values. The TPC model yielded an F-value of 8.70, indicating statistical significance, with only a 0.65% probability that the observed value is due to noise. Although extraction time and agitation speed did not show significant individual effects on TFC, the significant interaction between these two factors indicates that phytochemical extraction from *C. rotundus* relies on their combined influence. Agitation increases contact between the solvent and solid and reduces the boundary layer around the plant particles, while extraction time is sufficient to facilitate diffusion and movement toward equilibrium [21]. Thus, the synergistic effect of adequate time and proper agitation achieves the highest mass-transfer efficiency, resulting in the highest phenolics, flavonoids, and antioxidant activity recovered. The model's R^2 value is 0.8613, indicating a good fit, as it explains about 86% of the variability in TFC. An R^2 value of at least 0.80 is typically needed for the model to be considered a good fit for a bioprocess [5]. In this instance, the model is a good fit for the TFC extraction process, as the R^2 value exceeds that threshold. The good fit indicates that the model can be reliably applied to predict TFC under different experimental conditions of the extraction process, and hence it can be used for optimisation of the process with proper prediction. The high R^2 value and statistical significance indicate the model's reliability for assessing the influence of extraction time and agitation speed on flavonoid content.

Table 4. ANOVA table for TFC

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.055	5	0.011	8.70	0.0065	Significant
A: Extraction time	8.594E-006	1	8.594E-006	6.777E-003	0.9367	
B: Agitation speed	2.066E-004	1	2.066E-004	0.16	0.6985	
AB	3.336E-003	1	3.336E-003	2.63	0.1488	
A^2	0.028	1	0.028	22.14	0.0022	
B^2	0.038	1	0.038	29.86	0.0009	
R^2	0.8613					
Adj. R^2	0.7623					

The quadratic model was used to create the response surface plot showing the interaction between the two independent variables, extraction time and agitation speed, and the TFC of the extract. Figure 2(a) shows how TFC varies with extraction time and agitation speed. Figure 2(b) and Figure 2(c) show the one-factor plots of TFC as a function of extraction time and agitation speed, respectively. The TFC vs extraction time curve (Figure 2(b)) shows that TFC increases with extraction time up to 5 h, then decreases. This suggests an optimum extraction time for the highest TFC yield, and that longer times do not increase TFC. This reduction is likely due to increased oxidation of the extracted phytochemicals in the crude extract, as reported by [5]. The longer the extraction time, the more likely flavonoids are to be destroyed by factors that may negatively affect them during extraction (e.g., air, heat, light). Because of their high oxidation and thermal susceptibility, longer extraction time may reduce TFC by degrading the molecules and consequently reduce their bioactivity and production.

Similarly, Figure 2(c) shows that agitation speed has a positive effect up to 200 rpm, and then a negative effect on TFC. The decrease at higher agitation speed is due to degradation of flavonoid content in the crude extract according to [1]. These TFC behaviours are enhanced with process optimisation but can be lost with long extraction or under harsh conditions, as shown in recent studies. For instance, optimised ultrasound-assisted extraction (UAE) conditions extracted 43.71 ± 1.92 mg/g of *Centella asiatica* after 30 min of extraction, with a risk of decreased extraction efficiency with longer extraction time or higher energy exposure, because of the sensitivity of flavonoids [26]. In a similar study, the optimum UAE parameters for *Lactuca indica* leaves were a liquid-to-solid ratio of 24.76 mL/g, power of 411.43 W, ethanol content of 30 min, and 48.01 mg/g of TFC. Their method outperformed the traditional extraction method, and they noted that adding more input parameters may not increase yield beyond a certain threshold. The results, as well as recent studies, match this pattern: higher processing intensity and/or duration increases flavonoid recovery, but degradation may occur if processing intensity is too high and/or duration is too long. Agitation speed increases the rate of mass transfer between the solvent and the plant matrix. Further extraction is impeded when agitation slows because layers of flavonoids form around plant particles, creating a concentration gradient. Agitation speed ensures flavonoid

concentration in the solvent by keeping the solution well mixed, increasing overall yield. This study's findings are consistent with the reported optimum agitation speed of 204 rpm, where further increases in agitation speed did not increase extraction efficiency, and bioactive compound yields decreased. The data indicate that extraction time and agitation speed should be optimised to extract the highest amount of TFC, and that long extraction times and high agitation speeds negatively affect the stability of the extracted compounds.

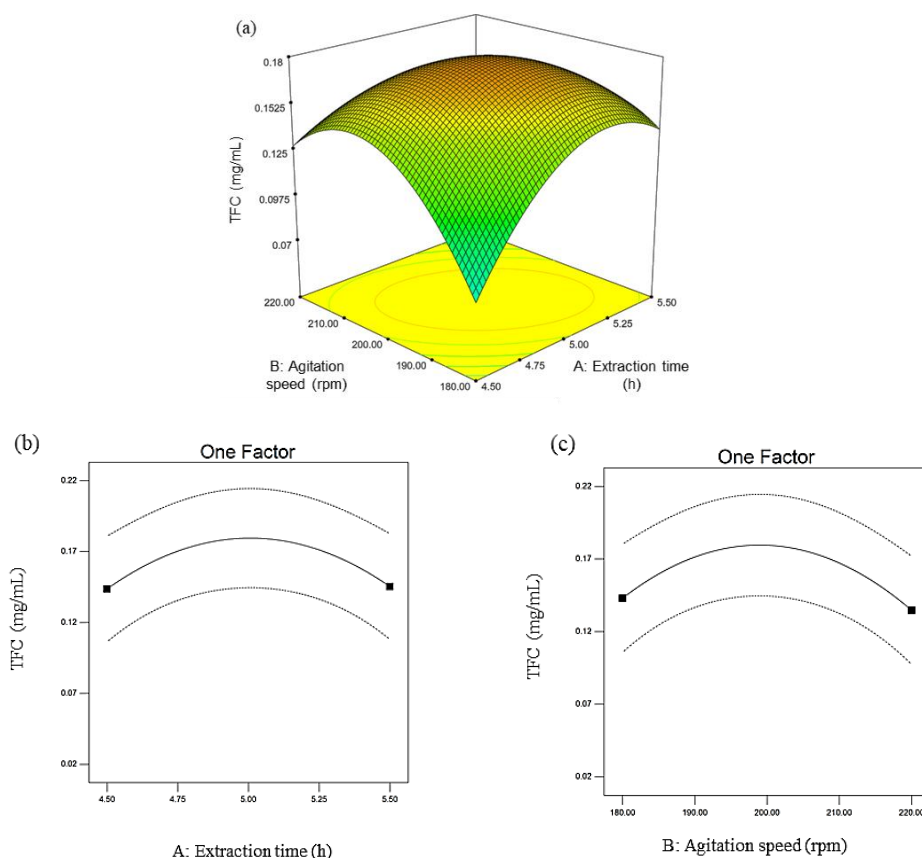


Figure 2. (a) 3D response surface of TFC as a function of extraction time and agitation speed, (b) effect of extraction time on TFC, and (c) effect of agitation speed on TFC

3.4 Data Analysis of AA on Design Expert

Table 5 presents the analysis of variance (ANOVA) for the AA model, where the model coefficients, independent variables, and possible interactions are evaluated. The model terms are significant because the “Prob>F” value is 0.0008, indicating that the factors in the model meaningfully affect the results. The significant model terms found in this study are AB (interaction between variables A and B), A^2 (squared term of variable A), and B^2 (squared term of variable B) with a p-value less than 0.05. In contrast, the p-values for factors A and B were high and were not significant. The TPC model produced an F-value of 17.55, indicating statistical significance and a 0.08% chance that the result was due to random noise.

Table 5. ANOVA table for TFC

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	5.786E-004	5	1.157E-004	17.55	0.0008	Significant
A: Extraction time	3.462E-005	1	3.462E-005	5.25	0.0557	
B: Agitation speed	1.486E-005	1	1.486E-005	2.25	0.1770	
AB	7.112E-005	1	7.112E-005	10.78	0.0134	
A^2	3.223E-004	1	3.223E-004	48.87	0.0002	
B^2	2.647E-004	1	2.647E-004	40.14	0.0004	
R^2	0.9261					
Adj. R^2	0.8733					

Individual effects of extraction time and agitation speed on AA were not significant, but the significant interaction between the two factors suggests that phytochemical extraction from *C. rotundus* depends on both factors. Increased solvent-solid contact and a thinner boundary layer around the plant particles enhance mass transfer, and sufficient extraction time allows effective diffusion and approach to equilibrium [21]. Thus, the synergistic effect of adequate hours and proper agitation provides the most efficient mass transfer, leading to higher extraction yields of phenolics, flavonoids, and antioxidant activity. The R^2 value of this model is 0.9261, which is a very good fit for the bioprocess model. As per [22], an R^2 value of at least 0.80 is desirable to confirm the model's adequacy. Thus, the high R^2 value in this study suggests that the model is a good representation of the extraction processes and the relationship between the independent variables and antioxidant activity.

The response surface plot from the quadratic model is shown, representing the interaction between AA and the other two variables. Figure 3 (a) shows the relationship between AA, extraction time, and agitation speed. Figures 3(b) and 3(c) show one-factor plots of the specific influence of extraction time and agitation speed on AA, respectively. Extraction time and AA are related such that AA increases with extraction time, peaks around 5 h, and then decreases. (Figure 3(b)). This suggests that increasing extraction time can improve AA, but that a maximum exists that could negatively affect it. This reduction in AA may be because phytochemicals in the crude extract are more likely to be oxidised, as reported by [10]. Over time, the extraction process reaches a balance, and the concentrations of AA in the plant material and in the solvent are in equilibrium. After this, increasing extraction time does not significantly improve the TFC, as most of the available AA has already been extracted. Therefore, continuing to remove compounds after the equilibrium point may be uneconomic and could cause degradation of the compounds. Similarly, the agitation speed of 200 rpm is seen to be the most effective in enhancing the activity of AA, as shown in Figure 3(c) above. This decrease in AA at higher agitation speed could be attributed to degradation of the antioxidant compounds present in the crude extract as reported by [27–28]. This is consistent with recent reports.

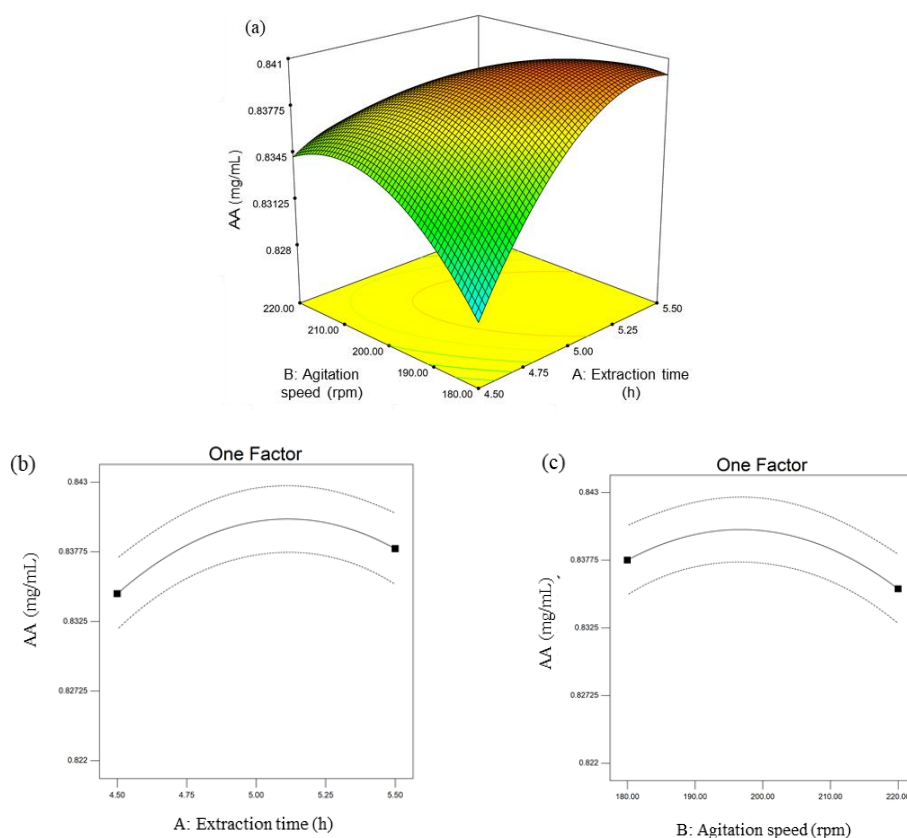


Figure 3. (a) 3D response surface of AA as a function of extraction time and agitation speed, (b) effect of extraction time on AA, and (c) effect of agitation speed on AA

For instance, [29] found that antioxidant potential (in terms of TEAC or IC_{50}) increased with the time or more severe method of extraction, but after the optimum, there was very little increase for reflux and Soxhlet extracts (IC_{50} = 0.37 to 0.39 mg/mL) and a slight decrease for reflux (IC_{50} = 0.37 to 0.30 mg/mL), suggesting that further extraction beyond the optimum point might result in the loss of some compounds. Similarly, [30] demonstrated that optimal ultrasonic-assisted extraction yielded high antioxidant recovery; optimal flavonoid-rich extracts had high DPPH activity and TFC of 48.01 mg/g and warned that extended extraction time or duration is not beneficial for increased antioxidant recovery. Increasing agitation speed enhances solvent mobility in the extraction system, improving interaction with plant cell walls. It changes cell structure, promoting better solvent penetration and, consequently, better flavonoid extraction. This mechanical motion aids plant tissue breakdown and reduces the time required for chemicals to diffuse into the solvent. Regarding agitation speed, [8, 31] found an optimum of 204 rpm for maximum phytochemical recovery and antioxidant response;

higher speeds decreased both due to mechanical and/or oxidative degradation. Overall, the findings underscore the importance of balancing extraction time and agitation speed to achieve the highest antioxidant activity while avoiding degradation or oxidation.

3.5 Optimisation and Results Validation

Design Expert software was used for the numerical optimisation method to optimise the extraction of phytochemical compounds from *C. rotundus*. The software optimised extraction time and agitation speed to maximise TPC, TFC, and AA and achieve optimum desirability. The software suggested an agitation speed of 194.75 rpm and an extraction time of 5.12 h. The desirability value of 0.926 shows the suitability of the proposed design parameters. For implementation, experiments were conducted at a rounded agitation speed of 195 rpm, which matches the precision of the equipment used. Table 6 shows the percentage error between the actual and predicted values, and the error is within a reasonable range. The predicted concentrations for the optimal conditions were 0.1394mg/mL for TPC, 0.1778mg/mL for TFC, and 0.8406mg/mL for AA, the highest of the three recorded. Thus, the best extraction time was 5.12 h, and the best agitation speed was 195 rpm to achieve the highest phytochemical yield.

Table 7 compares the yields of phytochemicals in this study with those of other studies using different extraction methods. The results indicate that the TPC (0.14 mg/mL), TFC (0.18 mg/mL) and AA (0.84 mg/mL) extracted from *C. rotundus* by maceration in an incubator shaker are lower than those reported for other plant materials extracted by more intensive methods like ultrasonic-assisted extraction (UAE), Soxhlet extraction and microwave-assisted extraction (MAE). For example, [32–33] obtained significantly higher yields with UAE, and [34–35] showed better extraction efficiency with Soxhlet and MAE methods, respectively. The lower TPC and TFC values in this study may be because the maceration process was mild; diffusion and mechanical disruption were used to release compounds from the sample [29]. Maceration does not involve cavitation, high temperature, or pressure, unlike UAE or MAE, and therefore does not achieve the same extent of mass transfer by breaking down cell walls, limiting the release of bound phenolic and flavonoid compounds [36]. Furthermore, the lack of high-temperature and high-pressure extraction conditions limits solvent penetration into the product and the solubility of phytochemicals, resulting in less efficient extraction [36]. The variation of yield may also be attributed to variations in plant matrix composition, with *C. rotundus* having phytochemicals that are more strongly bound to its cellular structure than other plant materials. Although the yield is lower, maceration remains a viable extraction process because it is easy, uses low energy, and requires less harsh chemicals. It's especially beneficial for storing thermolabile compounds that may break down at high temperatures or during intensive processing. Thus, although extraction efficiency is relatively low, the data generated in this study offer good insight into the phytochemical profile of *C. rotundus* under mild, environmentally friendly extraction conditions.

Table 6. The percentage error of the predicted and actual values of phytochemical content

Responses	Predicted value	Actual value	Error percentage
Total phenolic content (TPC)	0.1394	0.1399	0.35 %
Total flavonoid content (TFC)	0.1778	0.1788	0.56 %
Antioxidant activity (AA)	0.8406	0.8391	0.17 %

Table 7. Comparison of TPC and method with previous studies

Reference	Material	Method	Optimum Condition	TPC	TFC	AA
This work	<i>Cyperus rotundus</i>	Maceration in an incubator shaker	5.12 h extraction time and 195 rpm agitation speed	0.14 mg/mL	0.18 mg/mL	0.84 mg/mL
[32]	<i>Vitis vinifera</i>	Ultrasonic-assisted extraction	26.5 min extraction time, sonicator operating at 40 kHz	34.38 mg/g	10.21mg/mL	9.11mg/mL
[31]	<i>Licuala Spinosa</i>	Soxhlet extraction	90 min extraction time with agitation	9.42 ± 0.06 mg/g	8.96 ± 0.21 mg/g	0.032mg/mL
[30]	Lentil waste	Microwave-assisted extraction	5 min extraction time with agitation	28.3 ± 0.1 mg/g	1.89 ± 0.01 mg/g	0.39 µg/mL

4. Conclusions

This study examined the effects of extraction time and agitation speed on the phytochemical extraction of *C. rotundus*. The results demonstrated that AA was significantly affected by extraction time and agitation speed individually, as well as by their combined effect. In contrast, TPC and TFC were significantly affected only by the combined effect of extraction time and agitation speed, while their individual effects were not significant. Design Expert software 7.0.0 identified the best conditions as 5.12 h of extraction time and 195 rpm of agitation speed. Under these conditions, TPC, TFC, and AA were 0.1399 mg/mL, 0.1788 mg/mL, and 0.8391 mg/mL, respectively. These results are important for understanding how to maintain the pharmacological characteristics of *C. rotundus* during processing, which could improve product quality and facilitate commercialisation. Further, other potential factors that could affect extraction efficiency should be investigated to further optimise extraction yields from *C. rotundus*. Future work should integrate advanced extraction methods and explore a wider range of parameters to obtain marketable phytochemicals.

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Declaration of Competing Interests

The authors declare no identifiable competing financial interests or personal relationships that could have appeared to impact the study reported in this paper.

CRediT Author Contribution Statement

A.S. Baharudin: Investigation; Methodology; Analysis; Data curation; Writing – original draft; Visualisation.

N. Zainol: Conceptualisation; Writing – review & revise; Project administration; Supervision; Funding acquisition.

N. H. Aziz: Conceptualisation; Writing – review & revise

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

This study did not involve human participants or animal subjects. Ethical approval was therefore not required.

Generative Artificial Intelligence Disclosure

The authors declare that artificial intelligence (AI) tools, specifically large language models, were used only to assist with language editing, formatting, and improving the manuscript's readability. All scientific ideas, data analysis, interpretations, and conclusions presented in this work are the original work of the authors. AI did not influence the research design, data collection, results, or discussion.

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