



MICROWAVE-ASSISTED EXTRACTION AND HERBICIDAL POTENTIAL OF CINNAMIC ACID FROM CINNAMON USING RESPONSE SURFACE METHODOLOGY (RSM)

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Alao A.I., Adeoye B.K., Kalu S.O. (2026): Microwave-assisted extraction and herbicidal potential of cinnamic acid from cinnamon using response surface methodology (RSM). FUTA Journal of Engineering and Engineering Technology 20(1), 65-74

Received Date: 15.01.2026

Accepted Date: 11.03.2026

Abstract

Weeds reduce crop yields, and therefore, proper control measures are required and inevitable to control the actions of weeds on agricultural yield. The synthetic or man-made types of herbicides are mostly used and are hazardous to both human and environment. Thus, the need to develop natural herbicides. This study explored the use of Microwave-assisted extraction (MAE) technique for the extraction of cinnamic acid and reported the effects of the extraction parameters (ethanol percentage, microwave power, and extraction time), based on response surface methodology (RSM), on extraction yield. A microwave power of 160 W, 70.45% ethanol concentration and extraction time of 1 min resulted in a cinnamic acid yield of 8.66842 mg/ 100 mg cinnamon flour. The extracted cinnamic acid was able to show strong allelopathic character and wither the weed applied on within 5 days. MAE is an efficient technique for cinnamic acid extraction and provides the foundation for subsequent research about its application as a biodegradable herbicide.

Keywords: microwave-assisted extraction, allelopathic chemicals, green herbicide, cinnamic acid, cinnamon, response surface methodology.

Introduction

Weeds compete for all the available space and other resources and result in a yield loss of about 32%, a factor which is more damaging than that of pathogens at 15% (Van-Evert *et al.*, 2017). Such a large decline in yield can pose a threat to food security, and the availability of the agricultural produce is likely to reduce while the cost rises (Horvath *et al.*, 2023). Weed management needs to be well practiced to achieve food security and production.

Synthetic herbicides are widely used to kill weeds, and most applied twice annually (Parven *et al.*, 2025). Concerns about the harmful effects of synthetic herbicides like glyphosate on consumer health have grown because of their extensive use (Kaab *et al.*, 2019). Long exposure to artificial herbicides may result in residues in food items, which could eventually build up in the body and pose major health problems. Potential associations to cancer, hormone disruption, and other long-term

medical issues are among these effects (Hongoeb *et al.*, 2025). While this rationalizes and slows the efforts of identifying new natural compounds with novel herbicide target sites (Dayan *et al.*, 2012), medicinal and nutritional plant extracts have been considered the best pesticide reservoir due to their efficiency and environmentally friendly effects. These can help them support sustainable agriculture because they minimize yield loss due to weeds, which makes the food supplies more sustainable (Kaab *et al.*, 2019).

The cellular response of several weeds to multiple herbicides is becoming difficult to control as highlighted in the work of Manibharathi (Manibharathi, 2023). Therefore, there is a need to consider different allelo-rich plant extracts. These compounds are all characterized by strong allelopathic effects and can therefore find use as sustainable natural herbicides. Some of these allelochemicals are relatively large molecules which can suppress weed growth (Kong *et al.*, 2019).

Cinnamic acid, an allelochemical, has revealed its ability to suppress the growth of undesirable plants by using phytotoxicity (Mehmood *et al.*, 2018). According to Ranasinghe *et al.*, (Ranasinghe *et al.*, 2013), cinnamon is a widely known spice that contains various biologically active compounds like cinnamic acid and cinnamaldehyde which are mainly obtained from the cinnamon essential oil. Hence, owing to the prospect of utilizing cinnamic acid to act as a natural herbicide, this study aims to address the need for effective and sustainable weed management solutions from natural sources and will be relevant in supporting food availability. The identified possibilities may form the basis for the creation of effective natural herbicides, environmentally friendly and innocuous to the consumers, which are the urgent necessities for weed control.

Many researchers have reported the applicability of different plant extract for weed control due to their allelopathic characteristics and richness in phenolic compounds (Ribeiro *et al.*, 2015; Ximenez *et al.*,

2022). These extracts have shown high propensity and efficacy in replacing synthetic chemicals for weed control, and they are mostly produced from agricultural waste (Ribeiro *et al.*, 2015; Ximenez *et al.*, 2022).

Different methods have been applied for extraction of bioactive compounds in plants (Hossen *et al.*, 2023; Krumsri *et al.*, 2024; Spigno *et al.*, 2007; Ameer *et al.*, 2017a; Ameer *et al.*, 2017b). Comparing different extraction methods, MAE is noteworthy because it provides larger amount of bioactive compounds than reflux extraction (RE) (Ameer *et al.*, 2017a) and ultrasonic-assisted extraction (UAE) (Ameer *et al.*, 2017c). Microwave-assisted extraction applies microwave energy to heat the solvents and plant materials which enhances the cell breakdown and extraction yield (Ameer *et al.*, 2017b). This makes it a favorable method of extracting other compounds such as cinnamic acid from cinnamon (Ameer *et al.*, 2017b).

Table 1: Independent factors for the Box-Behnken design experiment for extraction of cinnamic acid

Variables	Symbols	Units	Range and Levels		
			Low (-1)	Medium (0)	High (+1)
Ethanol concentration	X ₁	%	25.00	62.50	100.00
Microwave power	X ₂	W	40.00	100.00	160.00
Extraction time	X ₃	min	1.00	3.00	5.00

Materials and Methods

Materials

Cinnamon (*Cinnamomum cassia*) powder was obtained from a supermarket in Akure, Nigeria. Uniform particle size of cinnamon powder was obtained using a sieve (Mesh No 40). The reagents used were of analytical grade.

Experimental Design

Table 1 shows the summary of the Box-Behnken design for the extraction of cinnamic acid from cinnamon. The ranges of independent process variables (ethanol concentration, microwave power and extraction time) were chosen based on preliminary experiments. The yield of total cinnamic acid was dependent variables (Y_n). Based on Box Behnken, a total of 17 experimental runs for MAE was carried out. The data was subjected to multiple linear regression (MLR) analysis, and the regression equation (Eq. 1) was obtained by analyzing the experimental data using SAS software (version 8.0, SAS Institute Inc., Cary, NC, USA) (Alao *et al.*, 2023).

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_1 + b_{13}X_1X_3 + b_{22}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

In this equation, Y_n denotes the response variable, and X_1 , X_2 , and X_3 denote the independent variables.

b_0 represents the constant term, and b_n is the regression coefficient for various terms, including the intercept, linear, quadratic, and cross-product terms (Alao *et al.*, 2023).

Statistical analysis was carried out to determine a suitable model to predict the experimental data obtained, using the ANOVA procedure as encompassed in the package. The optimum condition of the extraction process was determined through the aid of the numerical solutions in the software package (Alao *et al.*, 2023).

Extraction of Bioactive Compound

The microwave assisted extraction method (MAE) was conducted in accordance with the method reported by Lee *et al.* (Lee *et al.*, 2018). The extraction experiment was performed using the extraction conditions specified by Central Composite Design (CCD) with cinnamon extract from experiment as target response. For each run, an accurately weighed sample (2.5 g) was added to the extraction vessel and a total volume of 50 mL was made by adding extraction solvent (Lee *et al.*, 2018). At the end of each extraction, the vessel was cooled to room temperature and the extract was filtered through Whatman Filter Paper No. 41, and the

obtained extract was stored at 4 ± 1 °C for further analyses (Lee *et al.*, 2018).

Determination of Cinnamic Acid Yield

A 5 mL aliquot of extract was placed to an aluminum dish and dried at 105 °C until all solvent has been extracted (Lee *et al.*, 2018). A high-performance liquid chromatography (HPLC) (Agilent 1260, Agilent, Santa Clara, CA, USA) was used to analyze the cinnamic acid yield (Seo *et al.*, 2010). A 0.45 µm membrane filter was used to filter the sample, and a 20 µL injection volume was used in analyzing the sample (Seo *et al.*, 2010). A Zorbax Eclipse Plus

C18 column (4.6 x 150 mm, 5 µm) (Agilent Technologies, Santa Clara, CA, USA) was used at a temperature of 40 °C and a flow rate of 1.0 mL/min to separate the components of cinnamon aldehyde and cinnamic acid using HPLC (Seo *et al.*, 2010). A gradient flow with the mobile phase of 1% acetic acid (A) and 1% acetonitrile (B): A = 30:70 (40 min), B = 95:5 (0 min), at a specific wavelength of 280 nm, with a photodiode array detector was employed for the analysis (Seo *et al.*, 2010).

Table 2: Yield of cinnamic acid from cinnamon using microwave assisted extraction method

Std	Run	X ₁ : Eth. conc.	X ₂ : Microwave power	X ₃ : Extraction time	Cinnamic acid yield
		%	W	Min	mg/100 mg
3	1	100	100	5	8.34919
11	2	100	40	3	6.71501
7	3	62.5	160	5	6.30316
6	4	62.5	40	5	3.70261
5	5	62.5	100	3	6.72163
10	6	62.5	100	3	3.80227
4	7	62.5	100	3	7.33362
1	8	62.5	40	1	4.14631
14	9	25.0	100	5	3.73045
15	10	62.5	100	3	6.65381
9	11	62.5	100	3	8.52121
17	12	100	100	1	8.05808
12	13	25.0	40	3	8.00019
16	14	62.5	160	1	8.66842
13	15	25.0	100	1	7.21453
2	16	100	160	3	5.61012
8	17	25.0	160	3	7.93899

Results and Discussion

Efficiency of Microwave-Assisted Extraction Method

The cinnamic acid yield for each of the experimental run is reported in Table 2. The findings of this experiment can be discussed in connection with other investigations related to cinnamic acid extraction. Dinani *et al.* (Dinani *et al.*, 2018)

reported the isolation of cinnamic acid derivatives from *Allium tripedale* bulbs through normal solvent extraction procedures. It was observed that extraction yield was affected by both solvent concentration and extraction time, however the impact of temperature exceeded that of concentration of the solvent (Dinani *et al.*, 2018), which is similar to the trend observed in this study,

where microwave power (which is equivalent of temperature control) was found to be much more influential than concentration of ethanol.

The other comparative characteristic is the contribution of microwave power in the improvement of cinnamic acid extraction. Thi *et al.* (Thi *et al.*, 2017) focused on the synthesis and plant growth inhibitory activity of cinnamic acid derivatives and found out that cinnamic acid could be bioactive but if the extraction process is usually tended to high temperatures or long extraction time then the compound will degrade (Thi *et al.*, 2017). This finding agrees with the observation in the present study that short extraction times and high microwave power were the most desirable for achieving high yields of cinnamic acid without degradation.

Furthermore, Su *et al.* (Su *et al.*, 2019) also employed infrared-assisted extraction; the authors

established that using higher energy extraction techniques, which include microwave and infrared extraction procedures, favour the recovery of large quantities of cinnamic acid than conventional solvent extraction processes (Su *et al.*, 2019). The energy that was used by these methods increases the speed of extraction than the other conventional methods which are in congruence with this study since increased microwave power (160 W) and shorter extraction time (1 minute) yielded the best results. In the same way, Gilani and Najafpour (Gilani and Najafpour, 2022) observed that optimization of extraction parameters like temperature and time can enhance the yield of bioactive compound in agreement with the result yielded in this study (Su *et al.*, 2019).

Table 3: ANOVA for quadratic model

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	9.36	3	3.12	1.01	0.4178	not significant
A-Eth conc	0.4270	1	0.4270	0.1388	0.7155	
B-Power	4.44	1	4.44	1.44	0.2514	
C-Time	4.50	1	4.50	1.46	0.2479	
Residual	40.00	13	3.08			
Lack of Fit	27.93	9	3.10	1.03	0.5318	not significant
Pure Error	12.07	4	3.02			
Cor Total	49.37	16				

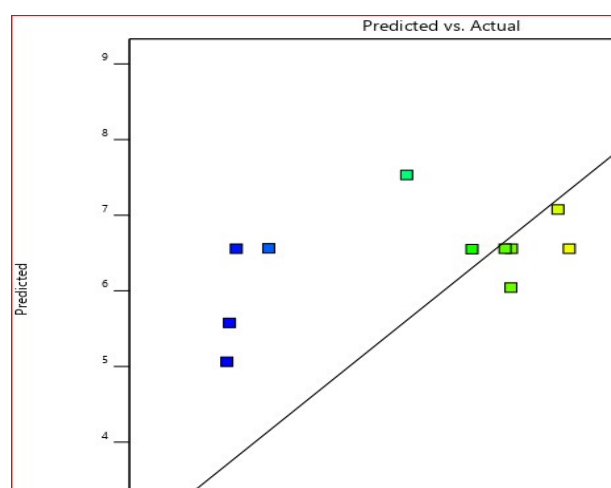


Figure 1: Predicted vs Experimental values of MAE of Cinnamic Acid

Extraction Variables and their Effects

The independent variables shown in Table 2 include ethanol concentration, microwave power, and extraction time; all three elements play a major role in deciding cinnamic acid extraction. Ethanol is a common solvent employed in the extraction of bioactive compounds from plants such as cinnamic acid due to its polarity (Lee *et al.*, 2024). This tells that the kind of solvent used in extraction process has a significant impact on the yield. For example, Gilani and Najafpour (Gilani and Najafpour, 2022) observed that ethanol concentration is a critical determinant of the solubility of phenolic compounds like cinnamic acid. However, the extent to which

solvent influences the extraction varies with the extraction technique and factors applied in the exercise (Lee *et al.*, 2024). However, in this experiment microwave power and extraction time were the two main factors that appeared to have a potential of accounting for why ethanol concentration is not a very sensitive factor when it comes to efficiency of extraction. These findings agree with the findings of Lee *et al.*, (Lee *et al.*, 2018) who pointed out that an optimal concentration of ethanol varies and should be adjusted to other attributes like temperature or microwave power.

Table 4: Summary of Fit Model

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.4178	0.5318	0.0027	-0.4601	Suggested
2FI	0.7228	0.4238	-0.1423	-1.6865	
Quadratic	0.8100	0.2446	-0.4342	-5.5087	
Cubic	0.2446		0.0217		Aliased

Table 5: Fit Statistics for Linear Model

Std. Dev.	Mean	C.V. %	R ²	Adjusted R ²	Predicted R ²	Adeq Precision
1.75	6.56	26.75	0.1897	0.0027	-0.4601	3.5134

Microwave-assisted extraction technique involves the use of electromagnetic energy to heat both the solvent and the plant matrix. In the work of Lee *et al.* (Lee *et al.*, 2018), it is revealed that an increase of the microwave power results in higher energy impact on the solvent and plant material giving a better extraction yield. This is observed in this study as an increase in microwave power (160 W) resulted in higher yield, as the highest applied microwave power gave the highest yield of cinnamic acid.

Another important factor is extraction time, with a proven effect on cinnamic acid yield. Long extraction time may either help to release the bioactive compounds or lead to their breakdown (Agrawal and Nirmal, 2024). In this study, smaller extraction times, especially in combination with high microwave power provide the highest yields of cinnamic acid. This is in concordance with the report of Su *et al.* (Su *et al.*, 2019) who suggested that shorter extraction time particularly when combined with technique such as infrared-assisted extraction yields more phenolic compounds because long extraction time destroys some of the phenolic compounds due to their sensitivity. In this study, the

extraction of cinnamic acid at 160 W for one min yielded the highest amount (8.67 mg/ 100g) of cinnamic acid, and followed the trend reported in literature (Lee *et al.*, 2018; Su *et al.*, 2019) where the high yield is related to the rapid extraction methods. The results also confirmed that ethanol concentration had a low impact in the yield compared to microwave power and extraction time, coinciding with the reports of Ameer *et al.* (Ameer *et al.*, 2017b) and Lee *et al.* (Lee *et al.*, 2018) using green extraction techniques.

Statistical Analyses and Model Fit

Table 3 shows the Analysis of Variance (ANOVA) of quadratic model. The obtained p-value and R² values shown in Table 3 proves that quadratic model cannot really predict the yield of cinnamic acid from extraction conditions. This is also presented in Fig. 1 which shows the disparity in the experimental and predicted data based on the quadratic model. This could be because of interference among the independent variables resulting in a situation whereby a different combination of the independent variables yields a different value of the dependent variable as hypothesized by nonlinear or higher

order models (Imaia *et al.*, 2021). In the present study, some of the operational parameters examined displayed non-linear or curvilinear relationship with the yield of the bioactive compounds, as evidenced by the requisite literature, including Mehmood *et al.* (Mehmood *et al.*, 2018) and Salvador *et al.* (Salvador *et al.*, 2013); although in microwave assisted extraction, the temperature and the power is not uniform all through the sample.

Table 4 shows the Summary of Fit for different types of models. The summary indicates that the linear and quadratic alone are incapable of representing the extraction process because the models are not good predictors of the response from the experiment,

based on their negative R^2 (Kuhn and Johnson, 2013). However, a linear model was suggested because of its positive Adjusted R^2 , though it is very low. The significantly low value of R^2 in the linear model of this study as shown in Table 5 indicates that the linear model is incapable of representing the extraction process likely due to the interference of the variables. A multivariate face-centered cube design (FCCD) reported by Chaw *et al.* (Chaw *et al.*, 2017) for a similar work, using germination inhibition as dependent variable while extraction time, agitation speed and solvent consumption were the independent variables, could be explored.

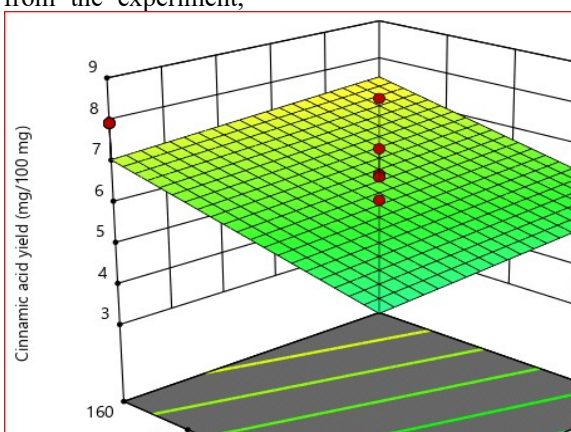


Figure 2: Interactive effects of ethanol concentration and microwave power on MAE yield of cinnamic acid.

Table 6: Equation derived for cinnamic acid extraction yield

In terms of Coded Eq.		In terms of Actual Eq.	
Cinnamic acid yield	=	Cinnamic acid yield	=
+6.56		+6.05640	
+0.2310	X_1	-0.006161	Eth conc
+0.7446	X_2	+0.012410	Power
-0.7502	X_3	-0.375121	Time

Lee *et al.*, (2018) also revealed that although RSM especially chose Box Benhken Design for enhancing extraction processes, higher modeling which includes interaction or cubic in this instance, might be needed in modeling complicated extraction. This complexity is due to the necessity to maintain the rate of energy input across the food mass in microwave power while at the same time considering the stability of bioactive compounds such as cinnamic acid (Lima *et al.*, 2021). In this study, there is no linear correlation of ethanol concentration with the yield of cinnamic acid, which

could probably be attributed to the solvent–matrix interactions not included in the present model.

Fig. 2 shows the interactive effects of the independent variables on the response (cinnamic acid yield). This shows that the interaction of all the variables does not significantly affect the extraction of cinnamic acid during the microwave assisted extraction, as shown in Table 4. As a matter of fact, only extraction time had a significant linear effect on the response. Potentially, fresh examination of these interactions employing other analytical models or experimental settings could help refine the

prediction of, and as well enhance, the production of cinnamic acid as proposed by López-González *et al.* (López-González *et al.*, 2023) in a metabolic study of the impact of cinnamic acid on plant roots.

Table 6 reports the coded and actual equations derived for cinnamic acid extraction yield. These equations could be employed to predict the extraction of cinnamic acid from cinnamon.

Viability of Cinnamon Extract (Cinnamic Acid) as Natural Herbicides

Cinnamic acid is a bioactive compound isolated from *Cinnamomum cassia* and has been predicted to

be a good natural herbicide because of high allelopathy, sufficient weed control, and less harm on environment. Mehmood *et al.* (Mehmood *et al.*, 2018) indicated that cinnamic acid can suppress the weeds, *Amaranthus retroflexus* and *Chenopodium album* because the extract retarded the root growth and nutrient absorption of the weeds. Furthermore, Salvador *et al.* (Salvador *et al.*, 2013) showed that cinnamic acid stimulated lignification in soybean root and decreased root elongation partially confirming its role as growth inhibitor.



Figure 3: Image of weed (a) before, (b) after day 1, (c) after day 2 and (d) after day 4; of applying cinnamic acid extract.

In this study, a maximum condition (160 W, 1 min, 70.45% ethanol) was used to produce 8.66842 mg/100 mg cinnamon flour. The potential of cinnamic acid as a natural herbicide was determined by applying about 10 cl of the optimum extract on a tropical weed (nodeweed - *Synedrella nodiflora* L). Cinnamic acid was able to exhibit allelopathic properties in which the overall activity of all other adjoining growths such as those illustrated in Figure 3 was suppressed due to interrupted metabolic processes of the target organisms (Palanivel *et al.*, 2021).

Conclusion

The extraction of cinnamic acid from *Cinnamomum cassia* by means of microwave-assisted extraction

was successful, and higher microwave power and shorter extraction time were shown to be more efficient. This research discovered that MAE at high microwave power (160W), and a shorter extraction time of 1 minute offered the highest concentration of cinnamic acid. This work has also shown that microwave power has the highest impact on the yield of cinnamic acid, while the concentration of ethanol has the second order importance. The effects of allelopathy of cinnamic acid prove that it is an effective natural herbicide thus providing a better alternative to synthetic chemical herbicide that is environmentally adversative through polluting the environment and cultivation of plants resistant to various herbicides. This research is relevant to the growing literature on natural herbicides and

demonstrates the application of cinnamic acid as an effective natural agent in the control of weeds and as the successful continuation of effective and eco-friendly bioactive compounds in agriculture. This goes further to show how cinnamic acid as natural bioherbicide can be commercially produced with speed using this energy intensive method.

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