

Research Article

Optimization of Essential Oil Extraction from Bitter Leaf (*Vernonia Amygdalina*) by Using an Ultrasonic Method and Response Surface Methodology

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Bitter leaf (*Vernonia amygdalina*) is a common bush or small tree that grows in tropical Africa. In the Ethiopian highland, the bitter leaf has been classified by the farmer as a versatile tree with high biomass yield and easy propagation. It is also well known in traditional medicine and nutritional use. The objective of this study was extraction and optimization of essential oil (EO) from the bitter leaf by using the ultrasonic extraction method and response surface methodology. The experiment was designed by Box–Behnken Design (BBD) with three factors to investigate the effect of sonication time (10 min to 30 min), ultrasonic power (100 to 200 W), and liquid-solid ratio (4 to 8 ml/g (ml of solvent per g of bitter leaf powder)). The significance of the process variables was analyzed using analysis of variance (ANOVA), and the quadratic model was fitted to the experimental results. Thus, the independent variables, sonication time, sonication power, liquid-solid ratio, and their interactions contributed a significant effect on the yield of extraction. As the result of RSM optimization, the best yield of EO was found at sonication time (17.263 min), sonication power (150.677 W), and liquid-solid ratio (6.811 ml/g). Experiments conducted under these conditions resulted in an EO yield of (4.185% g/g). The results exhibited that the RSM and BBD were effective for optimization of studied ultrasonic process variables for the maximum yield of EO from the bitter leaf (*V. amygdalina*).

1. Introduction

Tropical Africa is home to the common bush or tiny tree known as bitter leaf (*Vernonia amygdalina*). Bitter leaf is well known to cultivators in the Ethiopian highlands as a versatile tree with simple propagation, high yield, and great compatibility with other crops that do not compete with them for soil moisture or nutrients but instead aid in increasing the soil fertility and growth of perennial crops [1, 2]. Numerous phytochemical investigations have been carried out as a result of their therapeutic and dietary benefits [3]. The tannin, saponin, flavonoid, alkaloid, and terpenoid content of the bitter leaves has been determined using phytochemical analysis [4].

Significant levels of phenolic chemicals are present in the essential oil (EO) derived from the aerial parts, particularly

the leaves. Based on the concepts of distillation and mass transfer, numerous methods for extracting essential oils have been created [5]. The literature has reported on conventional methods such as steam distillation, solvent extraction, and hydrodistillation or water distillation. Finding more effective methods for the extraction of essential oils is necessary because existing methods have some drawbacks, including low extraction efficiency, lengthy extraction times, and substantial amounts of toxic solvent waste [6]. In contrast to traditional extraction methods, innovative extraction techniques have been created to increase extraction efficiency and yield, speeding up the extraction process, using less time, energy, solvent, facilitating mass and heat transfer, ensuring a safe and pure product, and reducing operational costs [5].

A potential method for extracting essential oils that is economical, straightforward, and effective is ultrasonic aided

extraction. Ultrasound's technical benefits are—mass transfer intensification, cell disruption, improved solvent penetration, and capillary action, high recovery yield, and quick extraction times—confirming that it is a suitable extraction technique for processing plant materials, especially compounds with low molecular weight [7–9].

Currently, response surface methodology (RSM) has been thoroughly investigated to analyze and optimize parameters influencing the ultrasonic-assisted extraction yield of bioactive components of plants and has demonstrated satisfactory results [7, 8, 10–13]; however, limited information was found on the application of RSM to the extraction yield of essential oil from bitter leaves (*V.amygdalina*) using ultrasonic assisted with methanol. Therefore, the goal of the study was to use RSM and Box–Behnken Design (BBD) in an experiment to identify the best-operating conditions (solid-liquid ratio, sonication time, and ultrasonic power) for the extraction yield of essential oil from the bitter leaf.

2. Materials and Methods

2.1. Plant Material. Bitter leaves (*V.amygdalina*) were collected from plants growing in Jimma, Ethiopia. The leaves were washed with distilled water and air-dried at room temperature for two weeks. The dried sample was ground into a fine powder (4 mm particle size) and packaged in a high-density polyethylene bag. The prepared sample was kept at 4°C until it was used.

2.2. Ultrasound Extraction Procedure. The extraction process was performed by using an ultrasonic bath (Elmasonic S60 H, Germany). Different solid-liquid ratios of (bitter leaf powder and methanol) were mixed; then, the mixture was soaked for 3 h before ultrasonic extraction as described in the method [14, 15] with a slight modification. Following soaking, the samples were subjected to ultrasonic treatment with an ultrasonic cell pulverizer at a constant ultrasonic frequency (25 kHz), a temperature of (25°C), various power levels, and sonication times. The temperature of the ultrasonic bath was kept constant by continuously pumping thermostatic water in and out. The extracts were filtered with Whatman No. 1 using a vacuum pump after extraction. The solvent was then vaporized from the extracts using a rotary evaporator at 40–50°C and low pressure. The extract was kept at –8°C until needed [14]. The yield of essential oil was calculated as follows:

$$Y = \frac{m_1}{m_2} \times 100\%, \quad (1)$$

where y is the yield of the bitter leaf essential oil % (w/w), m_1 is the mass of the extracted essential oil (g), and m_2 is the mass of the bitter leaf powder used (g).

2.3. Experimental Design. Using ultrasonic extraction, the response surface methodology (RSM) and BBD (by design expert 11.1.2.0 software) were used to achieve the highest yield of bitter leaf essential oil (EO). The ultrasonic power

(100, 150, and 200 W), sonication time (10, 20, and 30 min), and liquid-solid ratio (4, 6, and 8 ml/g) were chosen as independent variables (see Table 1). The yield of essential oil was the response variable. BBD determined 17 randomized experiments, including five replicates at the center points.

3. Results and Discussion

3.1. Response Surface Methodology Analysis. To maximize the yield from the extraction of essential oils, RSM and BBD were used to examine the individual and combined effects of the process variables (sonication time (min), ultrasonic power (W), and liquid-solid ratio (ml/g)). The design matrix of 17 runs was carried out by BBD, and the yield of essential oil was calculated as the ratio of extracted oil mass per mass of the bitter leaf powder used according to Equation (1); the observed results are presented in Table 2.

3.2. Model Fitting and Statistical Analysis. The experimental data were fitted using a second-order polynomial equation. The regression equation was expressed in terms of coded levels in Equation(2) as a function of the independent parameters, sonication time (A), ultrasonic power (B), and liquid-solid ratio (C). Three primary effects and a three-factor interaction make up the model. The negative sign of the coefficient indicates that as the level of the variable rises, the yield of essential oil falls, whereas the positive sign indicates that the yield rises. In contrast to the quadratic variables A^2 , B^2 , C^2 , and the variable interactions AB and AC , the quadratic model reveals that the linear variables B , C , and BC had positive effects on the extraction yield.

$$Y = 4.0956 - 0.048625A + 0.122375B + 0.4295C - 0.06925AB - 0.204AC + 0.111BC - 0.446425A^2 - 0.258425B^2 - 0.383175C^2 \quad (2)$$

3.3. Analysis of Variance (ANOVA). The significance of the regression coefficients and their validity are summarized in Table 3. The model had a statistical significance level of ($p < 0.05$). The F -value of 195.33 implies that the model is significant. There is only a 0.01% chance that an F -value this large could occur due to noise. The quadratic coefficients A^2 , B^2 , and C^2 as well as the linear coefficients for A , B , and C and the cross coefficients AB , AC , and BC were all statistically significant.

Additionally, the model performance was evaluated using the determination coefficient (R^2), adjusted (R^2), the coefficient of variation (CV), and adequate precision, which were computed and selected as auxiliary statistical metrics. The polynomial regression model suited the dependent variable values well, and the observed values were reasonably compatible with the anticipated value and the experimental data, according to both R^2 (0.9960) and adjusted R^2 (0.9909). Higher adjusted R^2 (0.9909) and anticipated R^2 (0.9476) values demonstrated the model's greater significance [13].

The lack-of-fit test was used to examine the model's "fitness" ($p > 0.05$) (Table 3), which demonstrated the

TABLE 1: Codes and levels of independent variables used in the response surface methodology (RSM) design.

Symbol	Independent variables	Coded levels		
		Low (-1)	Middle (0)	High (+1)
A	Ultrasonic power (W)	100	150	200
B	Sonication time (min)	10	20	30
C	Liquid-solid ratio (ml/g)	4	6	8

models' appropriateness to adequately predict the variation [7]. The inability of the model to accurately describe the data in the experimental domain, the points which were not included in the regression, is shown by a significant lack of fit ($p < 0.05$) [10]. The p value for the lack of fit in our investigation was 0.0649, demonstrating the model equation's suitability for yield prediction.

The coefficient of variation (CV) describes how dispersed the data are; a value less than 10 indicates that the model is reproducible, and the experimental results are precise and reliable [7]. The coefficient of variation (CV) obtained was 1.29%, indicating that the experimental data were reliable and the model was repeatable. With an "adeq precision" of 40.373, this model can be used to navigate the design space.

3.4. Diagnostics of Model Adequacy. It is crucial to assess whether the fitted model accurately approximates the actual values and exhibits a good fit. A diagnostic plot of predicted against actual values that are used to assess the model's applicability and demonstrate the relationship between expected and experimental data is shown in (Figure 1). The determination coefficient R^2 for the essential oil yield was 0.9960, showing that the response surface model in this study was appropriate for use in optimizing extraction variables because the experimental values were nearly in line with the predicted values [7].

3.5. Effects of Extraction Process Parameters on EO Yield. To understand how the variables interact and to establish the ideal level of each variable for the greatest response, the response surface curves were plotted [16]. Response surface methodology (RSM) used three-dimensional (3D) plots with the response as a function of two independent factors, while the remaining variables were kept at their constant values, to take into consideration the individual and interaction effects of independent variables on the EO yield. The 3D graphic response surface for the independent variable's impact on the EO yield is shown in Figures 2(a)–2(c).

Figure 2(a) demonstrates the overall influence of sonication time and ultrasonic power when the liquid-solid ratio was fixed at (6 mL/g). The yield was shown to increase with extraction time and ultrasonic power. However, it was discovered that after a certain value, the extraction yield begins to decline. According to the ANOVA results in Table 3, the interaction of sonication time and ultrasonic power was significant ($p < 0.05$). The influence of sonication time was negative, which means that as sonication time increased beyond the middle point (20 minutes), the EO yield decreased slightly. This could be caused by temperature

changes during the long sonication time, which destroys oils [17]. However, increasing the sonication power from (100 to 150 W) indicated an increase in the EO yield. At higher ultrasonic power, the cavitation bubble will burst quickly and the number of bubbles will increase, potentially resulting in more free radicals being formed in the aqueous solution [11]. By reacting with the EO constituent, these byproducts influence the yield degradation rate. Furthermore, the increased ultrasonic time and power may increase the likelihood of EO decomposition and potentially increase solvent loss through vaporization [8].

Figure 2(b) depicts the effect of the sonication time and the liquid-solid ratio on the EO yield when ultrasonic power was fixed at (150 W). The interaction of the sonication time and the liquid-solid ratio was found to be positively significant using AVOVA, with a p value of (<0.0001). Furthermore, the response plot revealed that as the sonication time and the liquid-solid ratio increased, the EO yield increased. However, the EO yield was observed to decrease beyond the center point. As demonstrated by the p value of (<0.0001) on the EO yield, the single impact of the liquid-solid ratio was greater than that of the sonication time. However, when the liquid-solid ratio is low, the difficult diffusion of solution results in low EO extraction efficiency [13]. Higher liquid-solid ratios, on the other hand, would result in a decrease in ultrasonic adsorption of the bitter leaf powder, resulting in insufficient power in facilitating cell wall breakage for the release of the essential oil compounds [18]. As a result, it is reasonable to conclude that there is always an optimal liquid-solid ratio to achieve the best results. The maximum yield (4.121% g/g) was obtained in this study at a liquid-solid ratio of 6 ml/g and a sonication time of 20 minutes.

Figure 2(c) illustrates that with a fixed sonication time of 20 minutes, the extraction yield initially increased with both the liquid-solid ratio and the ultrasonic power, but after a certain point, the extraction yield decreased for both variables. The interaction effect of the liquid-solid ratio and the ultrasonic power on the EO yield was determined to be considerably positive ($p < 0.05$) based on the ANOVA results. It can be shown that raising both variables while maintaining a constant sonication period enhanced the yield. Increased bubble formation and cell wall disruption brought about by high ultrasonic power resulted in increased solvent penetration, increased cell component release into the solvent, and improved mass transfer [19].

However, when the ultrasonic power and the liquid-solid ratio exceeded 150 W and 6 ml/g, respectively, the yield decreased. This could be because the increased ultrasonic power prevented the solvent from maintaining contact with the transducer surface [11].

3.6. Response Optimization and Model Validation. Based on the model, numerical optimization was used to determine the optimal combination of extraction process variables for the maximum EO yield. The desirability function was used to identify the optimum levels of factors. With a desirability value close to one, the optimal conditions were a sonication time of (17.263 min), a sonication power of (150.677 W), and a liquid-solid ratio of (6.811 ml/g). Under these conditions,

TABLE 2: The design matrix of BBD and yield of essential oil.

Run number	Independent variables			Response
	A: sonication time (min)	B: ultrasonic power (W)	C: liquid-solid ratio (ml/g)	Y: yield of essential oil % (g/g)
1	20	200	8	4.085
2	20	150	6	4.121
3	20	150	6	4.114
4	20	150	6	4.102
5	10	200	6	3.631
6	20	100	8	3.673
7	10	100	6	3.193
8	30	150	4	2.96
9	30	200	6	3.45
10	20	150	6	4.053
11	10	150	4	2.704
12	30	150	8	3.42
13	20	150	6	4.088
14	20	200	4	3.013
15	20	100	4	3.045
16	30	100	6	3.289
17	10	150	8	3.98

TABLE 3: Response surface model adequacy and analysis of variance (ANOVA) for a yield of EO.

Response	Std. dev.	C.V. %	R^2	Adjusted R^2	Predicted R^2	Adeq precision	Suggested model
Yield of EO	0.0464	1.29	0.9960	0.9909	0.9476	40.3731	Quadratic vs. 2FI
Source	Sum of squares	Mean square		F -value	p value		
Model	3.78	0.4200		195.33	<0.0001		Significant
A	0.0189	0.0189		8.80	0.0209		
B	0.1198	0.1198		55.73	0.0001		
C	1.48	1.48		686.43	<0.0001		
AB	0.0192	0.0192		8.92	0.0203		
AC	0.1665	0.1665		77.43	<0.0001		
BC	0.0493	0.0493		22.92	0.0020		
A^2	0.8391	0.8391		390.31	<0.0001		
B^2	0.2812	0.2812		130.79	<0.0001		
C^2	0.6182	0.6182		287.55	<0.0001		
Residual	0.0150	0.0021					
Lack of fit	0.0122	0.0041		5.59	0.0649		Not significant

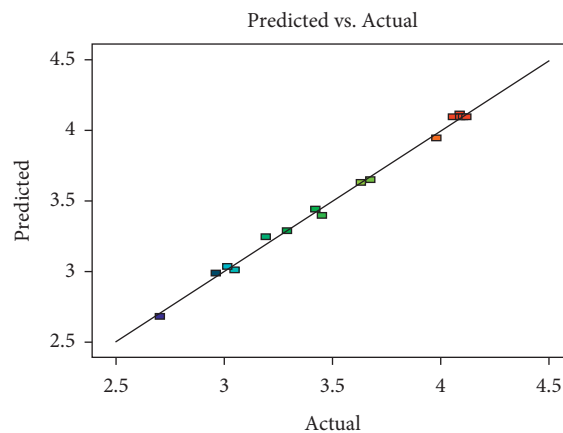


FIGURE 1: Diagnostic plot of model adequacy (predicated vs. actual).

the predicted EO yield was (4.212% g/g). In order to confirm the validity of the RSM model results, experiments were carried out under the optimum process conditions predicted

by the model, and the average EO yield obtained was (4.185% g/g). The data proved that the model designed in this study was valid, and no significant differences ($p > 0.05$)

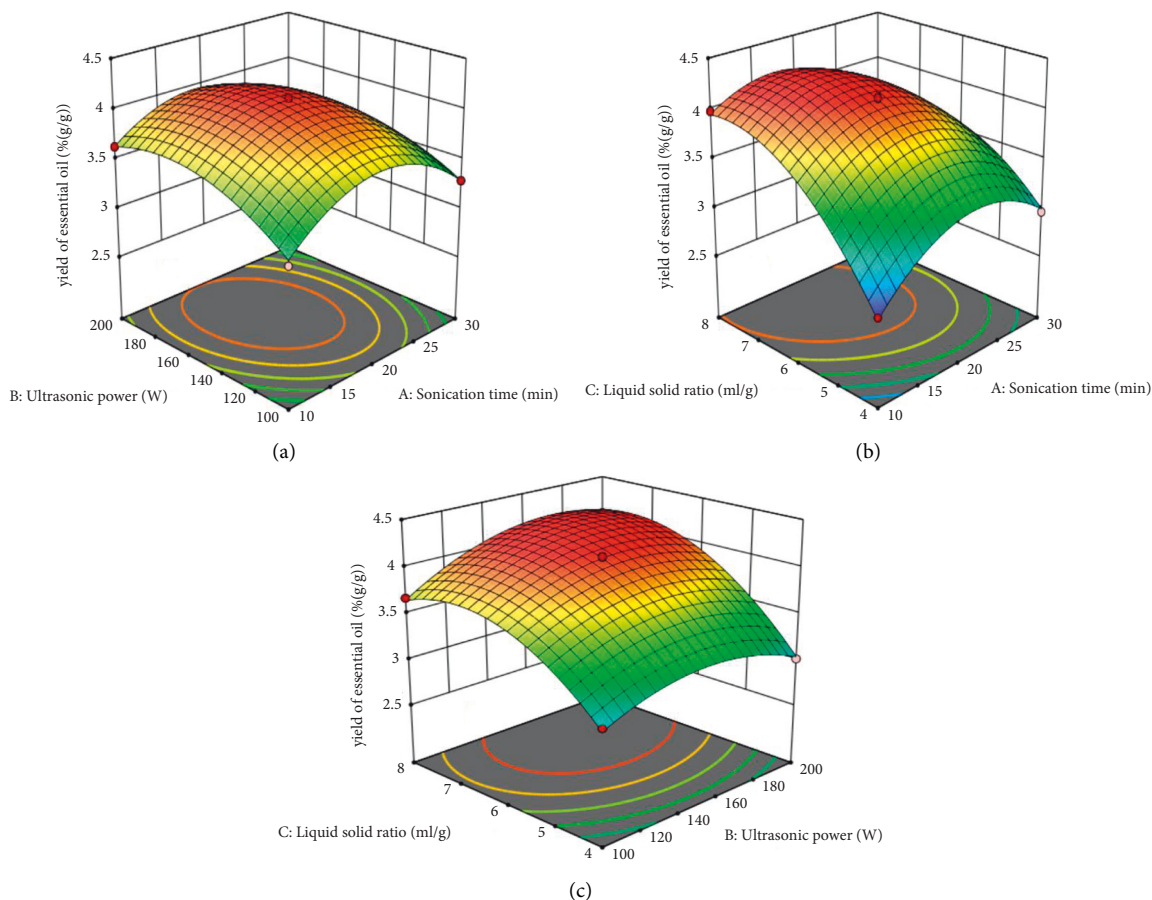


FIGURE 2: 3D response surface plot of the yield of EO: (a) effects of sonication time and ultrasonic power at the constant liquid-solid ratio, (b) effects of sonication time and the liquid-solid ratio at constant ultrasonic power, and (c) effects of ultrasonic power and the liquid-solid ratio at constant sonication time.

between the actual and predicted values were found [16]. Thus, BBD is effective to optimize the process parameters that affect the yield of EO extraction. To the best of our knowledge, this is the first attempt to optimize the conditions of ultrasonic extraction from the bitter leaf (*V. amygdalina*).

4. Conclusions

In this study, the RSM design was used to investigate the optimum operating conditions that could obtain a maximum yield of essential oil from the bitter leaf (*V. amygdalina*) when the ultrasonic extraction method with a methanol solvent is used. Based on analysis of variance (ANOVA), the independent variables, sonication time, sonication power, liquid-solid ratio, and their interactions contributed a significant effect on the yield of extraction. The polynomial regression model obtained fitted the dependent variable values well with R^2 (0.9960), which confirmed the observed values were reasonably consistent with the predicted value and the experimental data. The optimal operating conditions were sonication time (17.263 min), sonication power (150.677 W), and liquid-solid ratio (6.811 ml/g). Under this condition, the

experimental extraction yield of EO was (4.185% g/g). Our result verified that the RSM and BBD were effective for optimization of ultrasonic process variables for the maximum yield of EO from the bitter leaf (*V. amygdalina*). Furthermore, studies need to be conducted on scale-up and investigation of other ultrasonic process parameters on the yield of bitter leaf essential oil and the antioxidant activity of different treatment conditions.

Data Availability

Data are included within the article and supplementary material.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

All authors conceived, designed, and performed the experiments and wrote this paper, and they also participated in

experiment design and research supervision. The authors read and approved the final manuscript.

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