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Amaranthus dubius seeds oil extraction using green solvent approach with terpenes

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ABSTRACT

This research will explore the potential for utilizing wild amaranth (*Amaranthus dubius*) seeds, which are abundant and have not been utilized in Indonesia. This research will focus on optimizing the amaranth seed oil extraction process using the response surface method and the characteristics of the oil produced from amaranth seeds. The solvent used in this research is a biobased green solvent, terpenes. This research aims to explore the potential of utilizing wild amaranth seeds by optimizing the oil extraction process using a biobased green solvent, terpenes, through the response surface methodology (RSM). Fourier transform infrared spectroscopy (FTIR) results indicate that the terpenes solvent contains various terpenoids such as α-pinene, β-pinene, and camphene. By applying ultrasound-assisted extraction (UAE) and optimizing the critical process parameters (liquid-to-solid ratio, extraction temperature, and extraction time) through a Box-Behnken RSM design, the maximum oil yield was achieved under environmentally friendly conditions. The optimum condition was reached at a solvent-to-solid ratio of 5.13 ml/0.1 g, a temperature of 49.39 °C, and an extraction time of 47 minutes. With these optimal parameters, the predicted oil yield was 2.04%. The optimized extraction not only delivered a high-quality oil-rich extract but also minimized solvent residue, energy consumption, and hazardous emissions.

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1. INTRODUCTION

The sustainable development goals (SDGs) emphasize the critical need for environmental protection, poverty eradication, and food security for all [1]. These goals highlight the increasing demand for vegetable oils produced through environmentally friendly processes that utilize efficient, clean technologies. Vegetable oils are not only essential for food safety but also play a crucial role in pharmaceuticals, solvents, cosmetics, and oleochemical industries [2].

Southeast Asia is renowned as a leading producer of vegetable oils, particularly palm oil and coconut oil [3]. However, there exists a wealth of untapped potential in other oilseeds that can be transformed into fine chemicals. Despite their abundance, oilseeds in Southeast Asia have not been fully exploited. The *Amaranthus* genus, part of the *Amaranthaceae* family, includes over 60 species, such as *Amaranthus dubius* or wild amaranth, as shown in Figure 1. Originally from the Americas, wild spinach has spread globally and is often seen as a weed despite its abundance and availability. It remains largely unused, although species like *Amaranthus cruentus L.* are commonly used for extracting spinach seed oil. *Amaranthus* has crude oil content ranging from 5.1%-7.7%, with the highest dominant fatty acid components being linoleic acid (36.7%), oleic acid (18.7%), and palmitic acid (19.1%), as well as squalene content is between 3.6%-6.1% [4]. The squalene content is much higher than other vegetable oils such as coconut oil (0.002%), peanut oil (0.03%), rice bran oil (0.3%), and olive oil (0.4%). Another study reported that pretreatment of *Amaranthus* seeds through a grinding process, separating the endosperm from the bran, resulted in a higher squalene yield of 14% [5].

Figure 1. *Amaranthus dubius*

Squalene is a high-value natural compound with significant applications in cosmetics, nutraceuticals, and pharmaceuticals due to its antioxidant and skin-protective properties [6]. While several oilseeds are known for squalene content, *Amaranthus dubius* stands out as a promising underutilized source, especially given its high squalene yield relative to seed oil and its adaptability to tropical climates, making it a sustainable candidate for local-scale cultivation in Southeast Asia. Traditionally, oil extraction has been performed mechanically, but chemical extraction methods offer significantly higher efficiency [7]. In chemical extraction, the choice of solvent and extraction method plays a pivotal role in determining oil yield. At the beginning of the development of chemical extraction of oil, the solvents commonly used were chloroform and methanol [8]. However, n-hexane has become the predominant solvent due to its high yield and purification capabilities. Unfortunately, n-hexane is classified as a pollutant by the U.S. Environmental Protection Agency [9], necessitating the exploration of alternative, environmentally friendly solvents. Modern extraction techniques, such as ultrasound-assisted extraction (UAE) [10], pressurized liquid extraction [11], supercritical fluid extraction [12], and microwave-assisted extraction [13], have demonstrated superior results compared to conventional methods.

Recent advancements have led to the adoption of “green solvents” as alternatives to n-hexane. These solvents can be categorized into bio-based solvents, supercritical fluids, ionic liquids, deep eutectic solvents, and enzymatic extraction methods [14]. The extraction process is influenced by several parameters, including the solvent-to-solid ratio, temperature, and extraction time, all of which must be optimized to maximize oil yield [15]. Green solvents are derived from renewable resources and are designed to reduce environmental impact by minimizing waste and energy consumption. They are characterized by low toxicity, biodegradability, and the ability to be recycled [16]. Among these, terpenes have emerged as a promising class of green solvents. Terpenes, which are naturally occurring organic compounds found in plants, offer several advantages, including a high solvency power similar to n-hexane, a higher flash point, and a lower environmental footprint, aligning well with green chemistry principles [17]. Terpenoids derived from natural sources, such as p-cymene and α-pinene, which are contained in terpenes from pine trees [18], [19].

Most studies on terpenes as green solvents focus on terpenes extracted from essential oils, but their application in the UAE of oil from *Amaranthus dubius* remains unexplored. Furthermore, while squalene-rich oils from other sources (e.g., shark liver and olives) have been studied intensively, the use of terpenes to simultaneously optimize both oil yield and squalene extraction from underutilized *Amaranthus* species using response surface methodology (RSM) represents a novel approach. Indonesia is one of the largest terpenes oil-producing countries in Indonesia, so the industrial use of terpene oil as a solvent can still be beneficial. In research, terpene oil has a dissolving-like dissolving ability similar to n-hexane and a higher flash point, making it safer to use than other solvents that are widely used as bio-based solvents are cyclopentyl methyl ester, 2-methyltetrahydrofuran (2-MeTHF), and ethyl acetate [12]. Other green solvents, such as ionic liquids and deep eutectic solvents, are non-aqueous salts that melt under 100 °C, contain a cation and anion. Ionic liquid has no vapor pressure, hence no pollution. A deep eutectic solvent required hydrogen bond donor that is simpler and more economical to prepare than ionic liquids [20]–[22]. The integration of a locally abundant solvent with a locally available, underutilized biomass resource (wild amaranth) creates a circular, low-waste production system, enhancing both economic and environmental sustainability.

This research used terpenes as a green solvent for the extraction of oil from wild amaranth seeds. Terpenes, derived from natural sources such as pine trees, offer a sustainable and environmentally friendly alternative to traditional solvents. Indonesia, being one of the largest producers of terpene oil, provides a unique opportunity to leverage this resource for industrial applications. Terpenes not only have a dissolving ability similar to n-hexane but also possess a higher flash point, making them safer to use.

This research aims to explore the potential of utilizing wild amaranth seeds by optimizing the oil extraction process using a biobased green solvent, terpenes, through the RSM. By focusing on the optimization of extraction parameters and the characterization of the oil produced, this study seeks to contribute to the development of innovative and sustainable food and cosmetic products derived from local resources. The findings are anticipated to provide a sustainable alternative to conventional solvents, promoting environmental sustainability and economic growth.

2. RESEARCH METHOD

2.1. Raw materials

Flowers and seeds of *Amaranthus dubius* were handpicked from Yogyakarta Province. The collected flowers and seeds were air-dried under natural sunlight, and the seeds were subsequently harvested. The dried seeds were then ground with a mortar and pestle to produce a fine powder with a consistent particle size of 200 mesh. This powder was stored at −5 °C for preservation. The terpene as solvent was purchased from PT PPCI Pemalang, Indonesia.

2.2. Determination of solvent composition

The methodology for determining the composition of terpenes used as a solvent was using Fourier transform infrared spectroscopy (FTIR). Terpenes as a green solvent were subjected to FTIR analysis to identify functional groups and characterize the molecular structure. This technique provided insights into the presence of specific chemical bonds and functional groups within the oil, allowing for a preliminary assessment of its composition. The resulting chromatograms were analyzed to identify and quantify the individual bioactive compounds present in the terpenes, thereby providing a comprehensive profile of the solvent's composition.

2.3. Ultrasound-assisted oil extraction

The *Amaranthus dubius* seed oil was extracted using a sonicator operating at 500 W and a frequency of 50 kHz in an ultrasound bath. During filtration, a filter paper was used to remove solids from the sample. A rotary vacuum evaporator was used to remove any solvent left at 90 °C. The yield of the *Amaranthus dubius* seed oil was calculated using (1), where W_1 is the mass of oil obtained from the extracted sample and W_2 is the mass of the seed sample.

$$\%\ oil\ yield = W_1/W_2 \tag{1}$$

2.4. Response surface methodology optimization design

RSM was utilized to identify the ideal conditions for oil extraction. Building on the findings from single-factor experiments, a Box-Behnken design (BBD) was conducted, incorporating three independent variables: A (solvent–solid ratio), B (extraction temperature), and C (extraction time), each evaluated at three different levels on 2 replications. The data gathered from these experiments were analyzed using a second-order polynomial equation. The response regression equation was developed based on this analysis. The data obtained from these experiments were analyzed using a second-order polynomial equation. The response regression equation was formulated according to (2).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \tag{2}$$

All experiments were conducted randomly to minimize the influence of systematic errors on the outcomes. Analysis of variance (ANOVA) was utilized to assess the statistical significance of the regression model, while the interaction effects of each variable were evaluated using the P value. A systematic exploration of these parameters was conducted using a 3-factor solvent-to-solid ratio, temperature, and extraction time at a 3-level approach, yielding a total of 6 degrees of freedom. These factors were found to significantly influence the yield, with comprehensive details provided for each factor and its corresponding levels, as presented in Table 1. The results were evaluated using Minitab software.

Table 1. Process parameter optimization design

Variable	Code	Level 1	Level 2	Level 3
Solvent to solid ratio (mL/0.1g)	A	4	6	8
Temperature (°C)	B	30	45	60
Extraction time (min)	C	30	60	90

3. RESULTS AND DISCUSSION

3.1. Determination of terpenes solvent composition

The terpenes composition of the solvent was determined through FTIR analysis, as illustrated in Figure 2. This analysis provides insights into the composition of terpenoids present in the terpene's solvent. FTIR result indicates that the terpenes solvent contains various terpenoids such as α-pinene, β-pinene and camphene. The FTIR spectrum of the terpenes' solvent displays absorption bands that are characteristic of camphene, a monoterpenoid compound. Prominent aliphatic C–H stretching vibrations of $–CH_2–$ and $–CH_3$ groups appear near 2,916 cm^{-1}, indicating the presence of alkyl chains typical of terpene hydrocarbons. A symmetric CH_3 deformation band around 1365 cm^{-1} supports a branched-hydrocarbon structure, while an aromatic/cyclic C–H bending band at ≈ 786 cm^{-1} reflects ring-type motifs, suggesting a cyclic framework. In addition, the infrared spectrum exhibits diagnostic bands associated with α-pinene functional groups: alkene moieties are identified by =C–H stretching, C=C stretching, and C=C bending vibrations, whereas alkyl groups contribute characteristic C–H stretching and bending absorptions. Collectively, these spectral features confirm that camphene—together with α-pinene-related functional groups—is a principal constituent of the extract when terpene solvents are employed, thereby contributing to its observed biological activity.

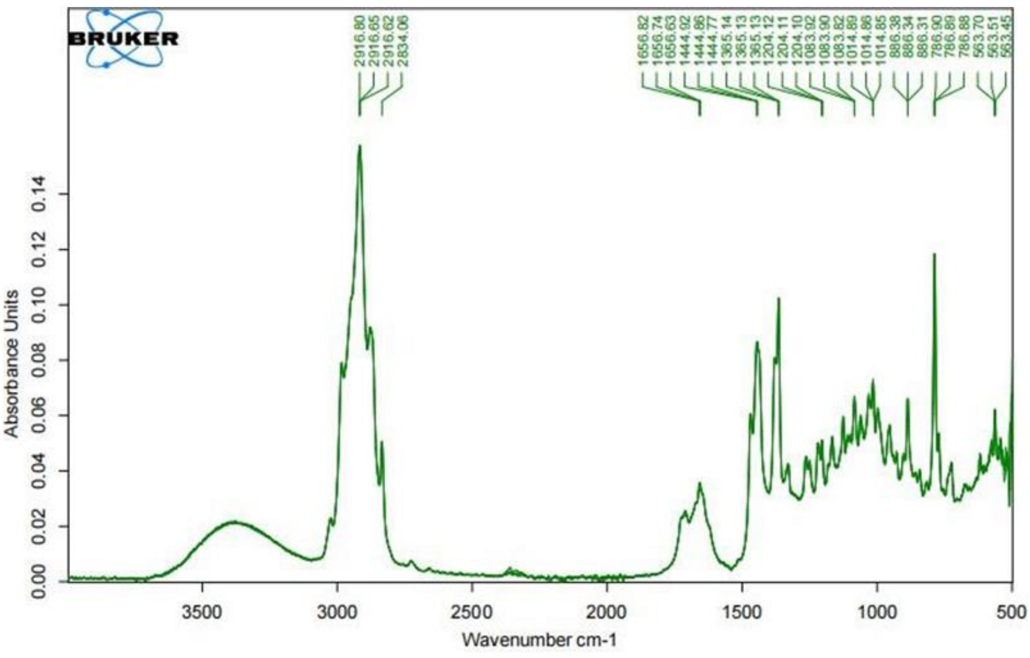


Figure 2. FTIR results of solvent

3.2. Optimization of ultrasonic-assisted *Amaranthus dubius* oil extraction by response surface methodology

3.2.1. Model fitting

The summary of the statistical models for the RSM using BBD optimization of the UAE process for *Amaranthus dubius* seed oil is presented in Tables 2 and 3. Based on the sum of squares of the model, a second-order polynomial was selected. The established model improved both the adjusted R^2 and the predicted R^2 values. Quadratic models were formulated based on the second-order polynomial, as specified in (3).

$$Yield\ (\%) = -6.72 + 0.634\,A + 0.1478\,B + 0.1486\,C - 0.0566\,A*A - 0.001427\,B*B - 0.001616\,C*C - 0.00152\,A*B + 0.00047\,A*C + 0.000018\,B*C \quad (3)$$

Table 2. Sequential model sum of squares

Source	Sum of squares	df	Mean square	F-value	p-value	Note
Mean vs total	21.80	1	21.80			
Linear vs mean	2.11	3	0.7048	3.94	0.0392	
2FI vs linear	0.0358	3	0.0119	0.0495	0.9844	
Quadratic vs 2FI	1.88	3	0.6261	58.39	0.0003	Suggested
Cubic vs quadratic	0.0484	3	0.0161	6.21	0.1417	Aliased
Residual	0.0052	2	0.0026			
Total	25.89	15	1.73			

Table 3. Model summary statistics

Source	Std. Dev.	R^2	Adj R^2	Predicted R^2	PRESS	Note
Linear	0.4229	0.5180	0.3865	0.0482	3.89	
2FI	0.4914	0.5268	0.1718	-6.7246	31.53	
Quadratic	0.1036	0.9869	0.9632	0.9029	2.44	Suggested
Cubic	0.0510	0.9987	0.9911		*	Aliased

3.2.2. Analysis of variance

To optimize the oil extraction from *Amaranthus dubius* using UAE, a BBD was implemented, and extraction yields were documented under different experimental conditions as shown in Table 4. The ANOVA results for the response surface polynomial model encompassing all independent variables are summarized in Table 5. The F values for this model were 29.03, and both P values were <0.0001, demonstrating that the models were highly significant. Additionally, a high coefficient of determination (R^2 = 96.32%) was observed, indicating a strong fit. The coefficients for total oil were utilized to formulate a final predictive equation, excluding any insignificant cross-terms. The regression equations for oil yield were found to be highly significant (P <0.05).

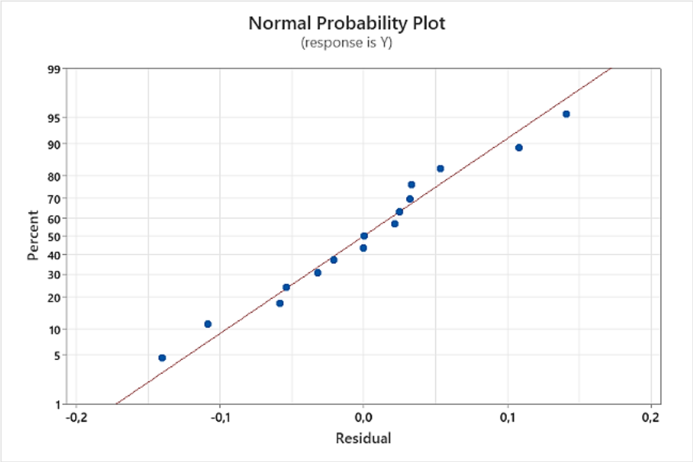
Table 4. BBD with the independent variables and response data: yield (%)

Run	Solvent to solid ratio (ml/0.1 g) A	Temperature (°C) B	Extraction time (minutes) C	Yield (%)	Yield (%) predicted
1	4	30	40	1.308	1.329
2	4	45	40	1.808	1.755
3	4	60	20	0.658	0.658
4	4	60	60	1.517	1.549
5	6	30	20	0.333	0.312
6	6	30	60	1.111	1.219
7	6	45	20	0.650	0.704
8	6	45	60	1.758	1.618
9	6	60	40	1.775	1.742
10	6	60	40	1.767	1.742
11	6	60	40	1.683	1.742
12	8	30	40	1.150	1.042
13	8	45	40	1.267	1.407
14	8	60	20	0.183	0.151
15	8	60	60	1.117	1.117

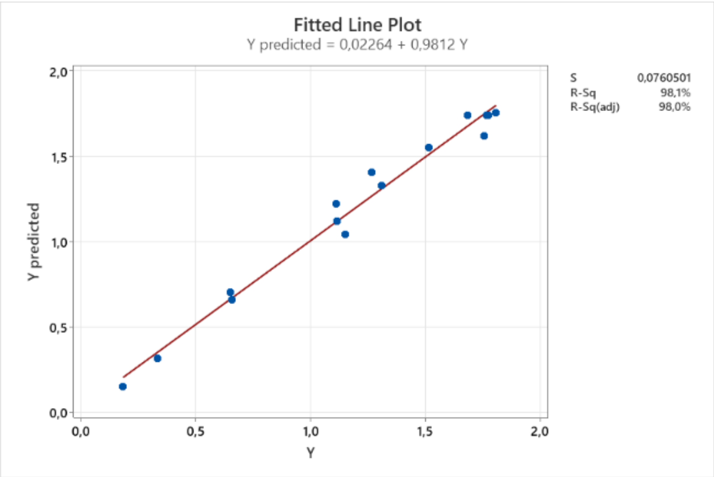
Table 5. Process parameter optimization design

Source	DF	Adj SS	F-value	P-value
Model	9	4.00450	29.03	0.001
Linear	3	2.17470	47.30	0.000
A	1	0.27638	18.03	0.008
B	1	0.27440	17.91	0.008
C	1	1.62391	10.96	0.000
Square	3	1.90794	41.50	0.001
A*A	1	0.18920	12.35	0.017
B*B	1	0.21577	14.08	0.013
C*C	1	1.54349	100.72	0.000
2-way interaction	3	0.01405	0.31	0.821
A*B	1	0.01248	0.81	0.408
A*C	1	0.00141	0.09	0.774
B*C	1	0.00017	0.01	0.921
Lack-of-fit		0.07149	9.27	0.099
Pure error		0.00514		

Figure 3 shows the validation of the data. Figure 3(a) displays the normal probability plot, this graphic indicates the evaluation of the regression model's normality assumption, whether the residuals from the regression model follow a normal distribution. The points on the plot closely follow a straight line, which indicates a strong alignment with normality assumptions, indicating that the model errors satisfy both the normality and identical distribution assumptions. The figure displays the plot of predicted versus actual response values, which helps verify the homogeneity of variances, a critical assumption for valid ANOVA results [23]. Moreover, Figure 3(b) shows the comparison between actual data and predicted values. The linear trend observed in Figure 3(b) confirms that the model effectively captures the relationship between predicted oil yield and actual measured values. Together, these graphical analyses play a vital role in evaluating the robustness, accuracy, and reliability of the model used in this study, providing valuable insight into the underlying relationships and model performance.



(a)



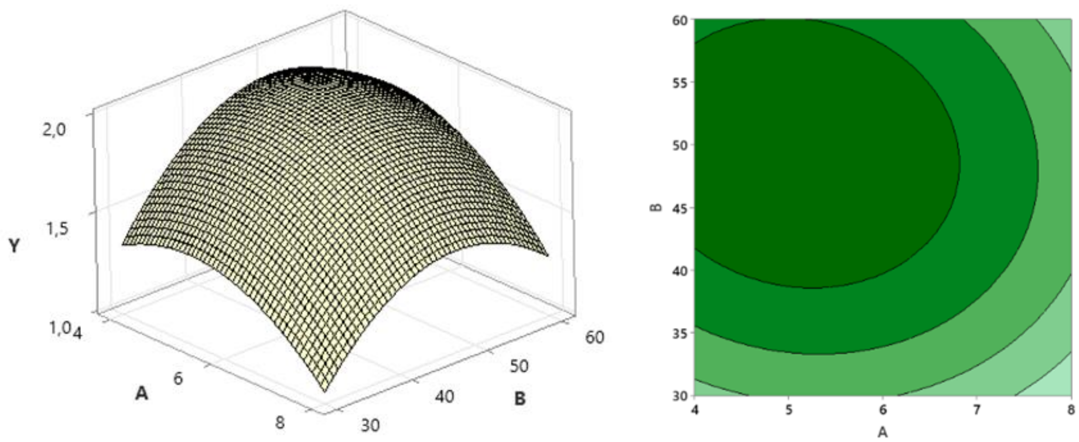
(b)

Figure 3. Plot of distribution: (a) normal probability plot and (b) predicted versus actual response plot

3.2.3. Impact of process variables on oil extraction

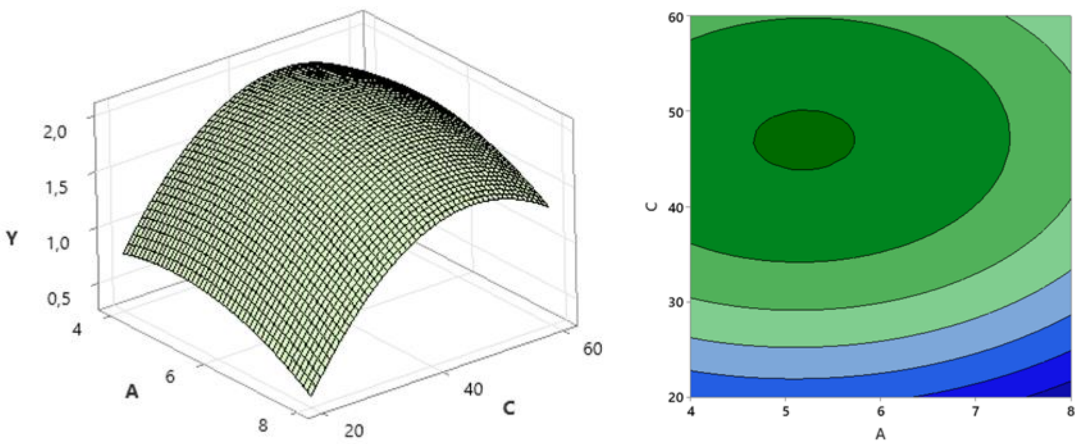
Three-dimensional (3D) response surface and two-dimensional (2D) contour plots were generated using the quadratic equation derived from the second-order polynomial model. Figure 4 presents the impact of the solvent-to-solid ratio and temperature on yield percentage; Figure 5 shows the effects of solvent/solid ratio and time on yield, and Figure 6 shows the effects of time and temperature on the yield. These visualization tools are essential for illustrating the interactions between process parameters and the corresponding experimental response. The 3D response surface plot provides a visual representation of the relationship between process parameters and the resulting of *Amaranthus dubius* oil yield as shown in Figures 4(a), 5(a), and 6(a), while the 2D contour plot offers a simplified 2D representation of the same relationship as shown in Figures 4(b), 5(b), and 6(b). These visualizations offer insights into the interaction between two parameters affecting the response, aiding researchers in analyzing and optimizing experiments to identify optimal conditions while reducing the need for extensive trial and error.

The effect of temperature on oil yield follows a downward-opening parabolic trend; yield rises from 30 °C to 45 °C and declines at 60 °C, likely because elevated sonication temperatures promote solvent evaporation and thermally induced degradation of target compounds [24]. The influence of extraction time exhibits a linear increase in yield between 20 and 45 min, then decreases, as prolonged exposure of the slurry to ultrasonic heating enhances mass transfer but also intensifies thermal degradation, thereby increasing apparent yield; however, excessively long extraction periods may surpass the optimum and reduce efficiency [25], [26]. The solvent-to-material ratio shows a positive linear relationship from 4 to 5 as shown in Figure 5, with higher ratios extending the contact time between solvent and matrix, resulting in significantly higher yields and allowing the response surface to approach its maximum. Elevated solvent ratios also augment ultrasonic intensity, promoting fragmentation of the material [27], [28]. In summary, temperature exhibits a bell-shaped relationship with yield, while solvent ratio and extraction time display positive linear correlations, indicating that increasing these parameters accelerates mass transfer from seeds to solvent.



(a) (b)

Figure 4. Effects of solvent/solid ratio and temperature on yield at 60 minutes in (a) 3D surface plot and (b) 2D contour plot



(a) (b)

Figure 5. Effects of solvent/solid ratio and time to yield at 45 °C (a) 3D surface plot and (b) 2D Contour plot

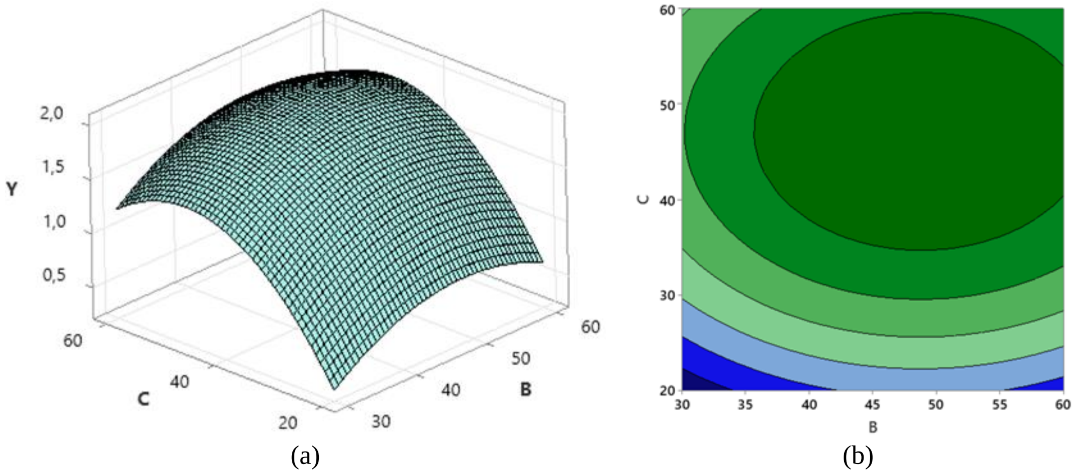


Figure 6. Effects of time and temperature on yield in (a) 3D surface plot and (b) 2D Contour plot

3.2.4. Optimization of oil extraction

The optimization of oil extraction process conditions was established using Minitab software, as shown in Figure 7. The highest yield of oil extracted from *Amaranthus dubius* was obtained under the optimal extraction conditions, solvent-to-solid ratio of 5.13 ml/0.1 g, temperature of 49.39 °C, and extraction time of 47 minutes. With this optimization, the predicted oil yield was 2.04%. Following this, experiments were carried out to confirm the predicted oil yield based on the optimal condition, with an average oil yield of *Amaranthus dubius* seeds of 1.92%. Although this value was slightly below the predicted yield of 2.04%, it remained in close agreement with the expected results. These results show a lower yield compared to using n-hexane as solvent, which was 4.36% [29]. Optimization of the extraction parameters through the BBD resulted in enhanced oil yield during UAE. The strong agreement between the experimental and predicted responses further validated the model's predictive capability and the effectiveness of the optimized conditions of oil extraction from *Amaranthus dubius* seeds using terpenes as a green solvent.

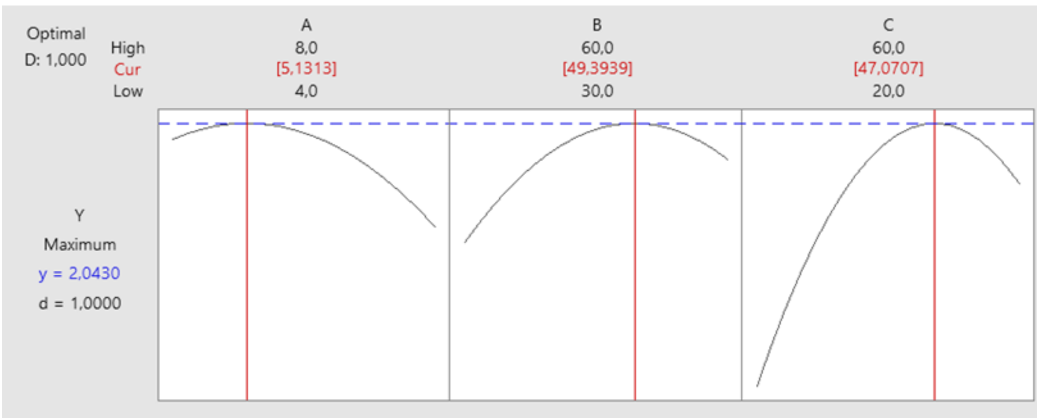


Figure 7. Optimization of *Amaranthus dubius* oil extraction

4. CONCLUSION

This study demonstrates that terpenes, renewable, low-toxicity bio-solvents derived from pine oil, can effectively replace petroleum-based n-hexane for extracting oil from wild *Amaranthus dubius* seeds. By applying UAE and optimizing the critical process parameters (solvent-solid ratio, extraction temperature, and extraction time) through a Box-Behnken RSM design, the maximum oil yield was achieved under environmentally friendly conditions. The optimum condition was reached at a solvent-to-solid ratio of 5.13 ml/0.1 g, a temperature of 49.39 °C, and an extraction time of 47 minutes. With these optimal parameters, the predicted oil yield was 2.04%. The optimized extraction not only delivered a high-quality oil rich in desirable fatty acids but also minimized solvent residue, energy consumption, and hazardous emissions.

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AUTHOR CONTRIBUTIONS STATEMENT

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C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

The author declares that there are no known conflicts of interest associated with this publication. There are no financial or personal relationships that could inappropriately influence or bias the content of this work.

DATA AVAILABILITY

The authors confirm that the data supporting this study are available within the article.

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