



Response Surface Methodology for Enhanced Recovery of Eucalyptus Pellita Essential Oil through Hydrodistillation

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Abstract

Essential oils (EOs) are valuable natural products with diverse industrial applications. The present study aimed to optimize the hydrodistillation (HD) process to maximize essential oil (EO) recovery of Eucalyptus pellita leaves. Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) was employed to model and analyze the effects of three key factors: extraction time (A), agitation speed (B), and solvent-to-leaf mass ratio (C). Seventeen experimental runs were conducted by using a Clevenger apparatus. The results showed that the ANOVA quadratic models were statistically significant for both mass and yield EO, with excellent coefficients of determination ($R^2 > 0.99$), adjusted R^2 , predicted R^2 , adequate precision, and non-significant lack-of-fit. All three linear parameters (A, B, C) and their interactions (AB, AC, BC) had a significant impact on extraction efficiency. Nonetheless, the quadratic terms (A^2 , B^2 , C^2) had a non-significant effect on the responses. The RSM predicted optimum conditions at 4.96 h, 874 rpm, and a solid-to-solvent mass ratio of 1:5.4 (g/g). This condition predicted 0.62-0.66 g of mass and 0.42-0.44% of yield EO. Experimental validation under these conditions produced an average EO mass of 0.635 g and an average yield of 0.4235%, both values closely matching the predicted outcomes. These results demonstrate the accuracy application of the RSM-BBD in optimizing HD for Eucalyptus pellita EO. The optimized parameters and validated model provide a practical and scalable framework for industrial EO production, supporting the transition from laboratory research to commercial implementation.

Keywords:

Eucalyptus pellita;
BBD;
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Optimization.

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1- Introduction

Essential oils (EOs) are complex mixtures of volatile compounds produced by plants and have been extensively investigated for their diverse biological activities, including antimicrobial [1-3], antioxidant [4-6], antiviral [7], and anti-inflammatory properties [8, 9]. Eucalyptus pellita is a notable EO-producing species, containing active compounds such as 1,8-cineole (eucalyptol), α -pinene, and limonene [10]. These compounds contribute to the distinctive aroma and the therapeutic potential of Eucalyptus EO, which is widely utilized in pharmaceutical, cosmetic, and botanical pesticide industries [10].

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Hydrodistillation (HD) is a conventional method for extracting EO from aromatic plants [11-13]. This process involves heating a mixture of plant material and water to generate vapor, which is then condensed and separated to yield EO [14]. The efficiency of HD is highly dependent on several process parameters, including temperature, extraction time, agitation speed, particle size, solvent type, and solvent-to-material ratio [10, 15, 16]. Slight variations in these operational conditions can lead to significant differences in both the yield and phytochemical profile of the extracted oil. Therefore, an optimization approach is crucial for establishing reproducible and efficient extraction protocols.

Response Surface Methodology (RSM) is an effective statistical approach for optimizing multiple factors simultaneously [10, 17, 18]. It enables the analysis of the relationship between process factors and the resulting responses through a structured experimental design, ultimately identifying optimal operating conditions [19]. The RSM model quantitatively evaluates both main effects and interactions among factors to understand their synergistic or antagonistic influences on responses. Experimental validation of the statistical model will be conducted, and the optimal conditions will be confirmed through runs at the recommended settings.

Numerous studies have investigated the application of RSM for EO extraction from Eucalyptus species. For Eucalyptus camaldulensis, researchers employed full factorial design (FFD), Box-Behnken Design (BBD), and Central Composite Design (CCD) [17]. BBD has also been used for Eucalyptus tereticornis [20], while CCD has been applied to Eucalyptus hybrid [18], Eucalyptus camaldulensis [21], and Eucalyptus intertexta [10]. However, FFD and CCD are not considered for experimental representations of EO extraction from Eucalyptus camaldulensis [17]. Therefore, BBD was selected for the present study.

Among the available designs in RSM, BBD is frequently selected for efficiency [17, 22, 23]. BBD uses only three levels for each factor (low, medium, and high), with experimental points placed at the centre of the design space rather than at the extreme corners [17, 22]. This feature makes BBD safer for processes sensitive to extreme conditions, such as solvent-based or thermal extraction [22]. Another advantage is the ability to generate reliable quadratic models with fewer experimental runs than other designs, such as CCD and factorial design, while still providing accurate predictions of outcomes [17]. BBD also facilitates a more comprehensive evaluation of interaction effects, such as the combined influence of extraction time and agitation speed on EO yield. Several previous studies have reported the successful application of BBD in optimizing the extraction of bioactive compounds, further emphasizing the relevance and applicability [17].

Although RSM has been extensively applied to various Eucalyptus species, there is a notable lack of research focused on Eucalyptus pellita. Specifically, the literature does not provide a statistically rigorous optimization of the HD process for Eucalyptus pellita. Factors that affect EO recovery in the HD process, like time, agitation speed, and solvent-to-leaf ratio, have not been investigated. To address the gaps, this study aims to optimize the HD process of Eucalyptus pellita EO using RSM-BBD. The findings are expected not only to enhance production efficiency but also to strengthen the potential industrial applications of Eucalyptus pellita EO.

2- Materials and Methods

2-1- Plant Material

Eucalyptus pellita leaves were obtained from a 5.4-year-old plantation located in compartment 22 A3 Toman 1 (03°38'21.5" S, 103°56'25.8" E) at an altitude of 70 meters, in Muara Enim Regency, South Sumatra Province, Indonesia. The plantation area receives a mean annual rainfall of 2395.2 mm, with an average temperature of 29.59°C, relative humidity of 42.4%, and soil pH of 4.2. The leaves were washed with clean water, cut into smaller pieces, and crushed to a particle size of 0.1–0.5 cm. The prepared samples were then used for HD.

2-2- Hydrodistillation Procedure

EO was extracted from Eucalyptus pellita leaves using HD with a Clevenger-type apparatus. The independent variables included extraction time (A), agitation speed (B), and solvent-to-leaves mass ratio (C). Other parameters, such as particle size and temperature, were maintained constant. Leaf particle size ranged from 0.1 to 0.5 cm, and the extraction temperature was set at 100 °C. The experiment was performed at a laboratory scale with an ambient temperature of 30±3 °C. For each run, 150 g of prepared leaves were combined with distilled water in a flat-bottom flask and subjected to the Clevenger-type apparatus according to the experimental design. The condenser outlet was connected to a Clevenger trap for oil collection. After distillation, the oil layer was separated, weighed on an analytical balance, and stored in amber vials at 4 °C until analysis. Each unique combination of factors (runs) was executed in triplicate.

2-3- Experimental Design

RSM-BBD was used in Design Expert® version 13 (Stat-Ease Inc., Minneapolis, MN, USA) to optimize the HD process [17, 24]. This design was selected for its efficiency in evaluating factor interactions with a relatively small number of experimental runs, while still providing reliable quadratic models [17]. The independent variables used were

extraction time (A), agitation speed (B), and solvent-to-leaves mass ratio (C), each at three levels: low (-1), medium (0), and high (+1), as shown in Table 1.

Table 1. Independent variable for RSM of the HD process

Code	Independent Variable	Level		
		-1	0	1
A	Extraction time (<i>h</i>)	3	4	5
B	Agitation speed (<i>rpm</i>)	300	600	900
C	Solvent-to-leaves mass ratio (<i>g/g</i>)	4	6	8

The responses observed were the mass and yield of EO. The response variable analyzed was the yield of EO (%), calculated based on the mass of EO obtained (g). To model and predict these responses, a second-order polynomial equation was used as illustrated in Equation 1 [19]. Where Y is the predicted response (mass or yield of EO), α_0 is the intercept, α_1 , α_2 , α_3 are the linear coefficients for A, B, C respectively, α_{11} , α_{22} , α_{33} are the quadratic coefficients for A^2 , B^2 , C^2 respectively, and α_{12} , α_{13} , α_{23} represent interaction effects between variables for AB, AC, and BC. A total of 17 runs were generated to develop a predictive quadratic model, and the resulting data were analyzed using analysis of variance (ANOVA) to assess the significance of each factor and the adequacy of the model [17]. The experimental design was generated from Design Expert software, as shown in Table 2.

$$Y = \alpha_0 + \alpha_1A + \alpha_2B + \alpha_3C + \alpha_{11}A^2 + \alpha_{22}B^2 + \alpha_{33}C^2 + \alpha_{12}AB + \alpha_{13}AC + \alpha_{23}BC \quad (1)$$

Table 2. BBD matrix with an average response for mass and yield of Eucalyptus pellita EO

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2
	A	B	C	Mass of EO (g)	Yield of EO (%)
1	4	900	4	0.57	0.38
2	5	900	6	0.64	0.43
3	5	600	8	0.62	0.413
4	4	900	8	0.56	0.37
5	3	900	6	0.48	0.32
6	4	600	6	0.56	0.37
7	3	300	6	0.55	0.367
8	4	600	6	0.55	0.367
9	4	600	6	0.54	0.36
10	5	600	4	0.59	0.39
11	4	300	8	0.62	0.41
12	5	300	6	0.54	0.36
13	3	600	8	0.56	0.37
14	4	300	4	0.48	0.32
15	3	600	4	0.47	0.313
16	4	600	6	0.544	0.363
17	4	600	6	0.55	0.367

3- Results and Discussion

Several process parameters strongly influenced the efficiency of HD, including extraction time, agitation speed, solvent-to-material ratio, solvent type, particle size, and temperature [10, 15, 16]. The contact time between the plant material and the extracting medium is a crucial part of the extraction process. Longer extraction durations facilitate more complete diffusion of essential oil components from the leaf matrix into the solvent or steam [18]. However, equilibrium is eventually reached between the plant material and the extracting media, defining an optimal extraction duration beyond which further extraction may not be appropriate. Prolonged extraction can degrade heat-sensitive components of the oil. A preliminary experiment was conducted by extending the extraction duration to 12-24 hours, with monitoring every hour. The results showed an increase in EO during the first 5 hours of extraction. After that, the EO produced reached equilibrium. These preliminary experiments served as the basis for selecting the extraction time limit of 3-5 hours.

The agitation speed directly influences the mass transfer rate. The boundary layer, also known as the film layer, is a thin, stationary layer of solvent that forms at the surface of any solid particle submerged in a liquid. Diffusion is prevented by this layer. Without agitation, compounds must diffuse slowly through this static layer. Agitation ensures all solid surfaces are continually exposed to the solvent, preventing dead zones where extraction would be incomplete. This encourages faster internal diffusion and prevents settling materials. While increasing agitation generally improves the efficiency of extraction, the relationship is not linear indefinitely. Too high agitation speed can cause problems, such as excessive heat, oxidation of sensitive compounds, foaming or emulsification, and mechanical degradation. There is an optimal speed. Based on references, the range of agitation speed was 300-900 rpm, with the optimum at 900 rpm [25, 26]. Another research used 0-400 rpm, with the optimum being 400 rpm, which produced a greater yield of EO [27]. Based on previous studies, 300 to 900 rpm was selected as the agitation speed limit.

The concentration gradient between the leaf surface and the solvent or steam is also affected by the solid/solvent ratio. Higher ratios increase the driving force for mass transfer by increasing the concentration of constituents in the medium. However, overly high ratios can cause saturation and result in inadequate extraction, thereby lowering extraction efficiency. The solvent would not have been able to extract enough essential oil from the leaves if there had been fewer leaves than the amount of solvent used. An important factor was the concentration gradient mass transfer between the liquid phase and the Eucalyptus leaves. According to Lainez-Cerón et al. [27], a solid-to-liquid ratio of 1:1 produced no EO, whereas 1:5 was optimum. Another researcher used a 2:1-6:1 solvent-to-leaf ratio, with the optimum at 4:1 [28]. Some researchers use a 10 times proportion of solvent to leaves to maximize the yield of EO [17]. Initially, a 10:1 solvent-to-leaf ratio was used, the same as our steam distillation parameter. This high ratio does not significantly affect the EO produced. In addition, there is reflux in the Clevenger apparatus used, so even if extraction takes a long time, it will not significantly reduce the amount of solvent and will not promote heat effects in the extraction. Based on these considerations described, a ratio of 4:1 to 8:1 was selected.

More significant interaction between the leaves and the media is generally enabled by a larger surface area, resulting in more effective essential oil extraction. The efficient transfer of EO from the bigger leaf to the solvent was limited by a smaller interfacial surface and the probability of a thicker cuticle [18]. In the impact of particle size on the yield of EO from plant material, it was essential to integrate initial findings with the plants' histological features. A limiting factor for endogenous secretory pockets (oil glands) of the schizogenous type (Eucalyptus species) is the degree of pulverisation [29]. The selection of leaf particle size range (0.1-0.5 cm) is based on research by [17, 28, 30]. They reported the optimum size for Eucalyptus camaldulensis is 0.5 cm [30] and 0.0375 cm [17]. Fine particle size of leaf (1-3 mm) showed increasing yield of EO compared to fibre cut and original size [28]. Preliminary experiments indicated that using leaves larger than 0.5 cm reduced extraction efficiency. In these trials, the larger leaf impeded vapor flow, trapping the steam and the volatile compounds it carried within the plant material, thereby preventing their full release and collection.

The temperature at which essential oils are extracted also significantly impacts their volatility. Elevated temperatures facilitate the release of volatile chemicals into the vapour phase for extraction by increasing the kinetic energy of the oil molecules, thereby promoting faster diffusion. On the other hand, lower temperatures could reduce extraction efficiency. There was no EO produced at a temperature below 100 C reported by [25], and only a little EO in the 80-90 C reported by Abed & Naife [30]. The optimum temperature to produce EO by the HD method was 100 C [25, 30]. Another research obtained 100-150 C, with the optimum at 120 C [28]. Elevated temperatures may increase extraction yield, but this approach carries significant trade-offs. It requires more energy and risks degrading heat-sensitive compounds such as monoterpenes and oxygenated monoterpenes, which are essential to the EO's aromatic and therapeutic profile. Therefore, maintaining the extraction process at water's boiling point of 100 °C offers an optimal balance. That can preserve both the chemical integrity and volatile quality of the extract while minimizing energy consumption.

The optimization of Eucalyptus pellita EO extraction using HD was modeled by using RSM. The experiment examined the effects of agitation speed (rpm), extraction time (hours), and solvent-to-leaf mass ratio on the responses, namely mass of EO and yield. The experimental design produced empirical results that were subsequently analyzed in depth. The regression models obtained were statistically significant, with high R² values, adjusted R², predicted R², and adequate precision values, and a non-significant lack-of-fit. The design of experiments (DoE) using BBD with 5 center points yielded 17 runs, as shown in Table 2.

The significance of the quadratic model for the mass and yield of EO was evaluated using ANOVA. Model adequacy was further assessed using several statistical parameters, including the significance of the model, the insignificance of lack-of-fit, coefficient of determination (R²), adjusted R², predicted R², and adequate precision [23]. Data instability was checked through data-fit predictive and actual graphs. Figure 1 demonstrates that for all responses, the predicted values were quite similar to the experimental values.

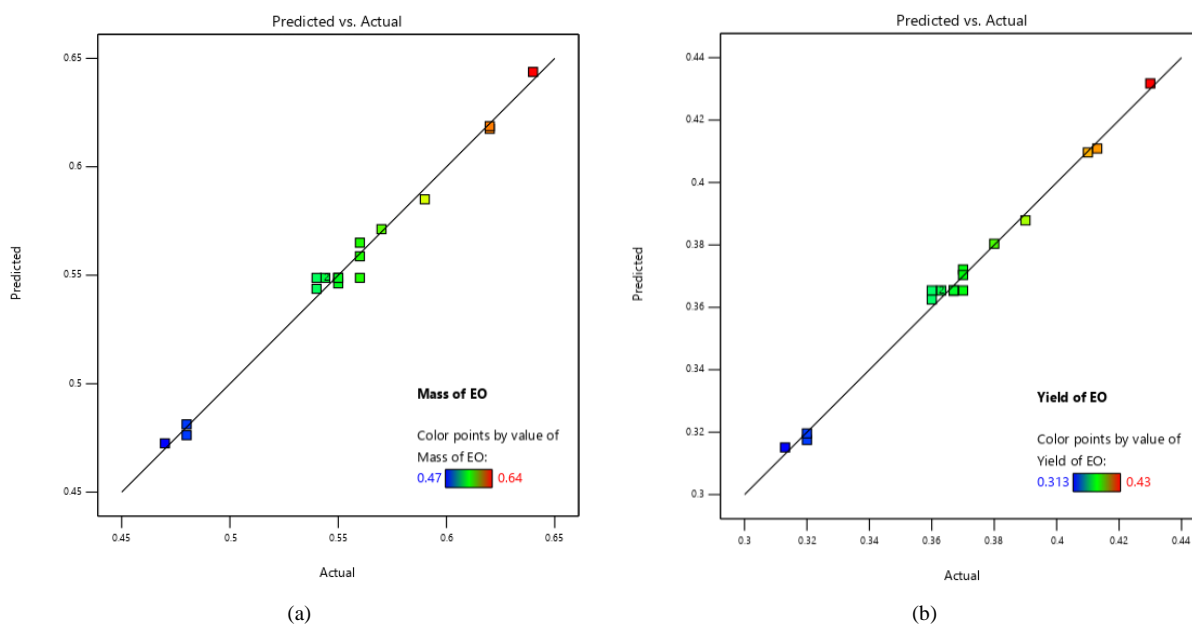


Figure 1. Data-fit prediction vs actual graphs for EO generated from the HD method: (a) mass, (b) yield

The Prediction vs. Actual graph for EO mass and yield shows the high accuracy of the RSM model in predicting HD results. The data points, colored to represent the variation in mass and yield of EO, are clustered along the diagonal line ($y = x$), indicating a strong fit and consistency between the model-predicted and actual experimental measurements. This confirms that the regression model effectively captures the relationship between process variables such as extraction time, stirring speed, and solvent-to-biomass ratio to the responses.

A statistical tool used to assess the importance of each term in the regression model is the p-value. In this case, it was used to assess how important the independent variables (extraction time, agitation speed, and solvent-to-leaf mass ratio) in predicting the selected responses (mass and yield of EO). A p-value of less than 0.05 was considered statistically significant, indicating that the corresponding term contributed significantly to the response's variability. In contrast, a p-value greater than 0.05 suggested that the term was not statistically significant and might not have a substantial impact on the response. A p-value ≤ 0.05 was considered statistically significant, with the following levels of significance applied: $p \leq 0.001$ (extremely significant), $0.001 < p \leq 0.01$ (highly significant), and $0.01 < p \leq 0.05$ (significant) [23, 31].

The lack-of-fit test was also performed to verify the models' suitability. It was also desirable to have an insignificant p-value for lack of fit, which measures the difference between the model's predictions and the actual experimental data points. This indicated that the model fit the data well and could accurately predict the responses within the designated range of input variables. It also showed that there were no systematic errors in the model and that it effectively captured the underlying relationships between the factors and responses. The sum of square values for all responses was low, indicating low variability. Additionally, the model's desirability was indicated by its lower degree of freedom; better responses for the model are indicated by mean square values closer to zero.

Table 3 shows that for ANOVA in the mass EO, the model F-value of 79.05 and p-value of < 0.0001 indicate that the model is statistically extremely significant. There is only a 0.01% chance that this magnitude of F will occur due to noise. A p-value of less than 0.05 indicates a statistically significant model. In this case, A, B, C, AB, AC, and BC are significant factors/variables. The quadratic terms (A^2 , B^2 , C^2) are statistically non-significant, as indicated by the p-value ≥ 0.05 . An F-value of lack-of-fit of 0.7284 implies that Lack of Fit is not significant ($p > 0.05$) compared to pure error. There is a possibility that 58.63% of the lack-of-fit F-value occurs due to noise.

For the ANOVA of the EO yield obtained by the HD method, Table 4 shows that the model is extremely significant, as indicated by an F-value of 126.02 and a p-value of < 0.0001 . The p-value is less than 0.05, suggesting that factors A, B, C, AB, AC, and BC are significant. The p-value of quadratic terms (A^2 , B^2 , C^2) is greater than 0.05, indicating non-significance. The value of 0.8115 indicates that lack-of-fit is insignificant compared to pure error. There is a 55.02% chance that the lack-of-fit F-value occurs due to noise.

Table 3. ANOVA quadratic model for the mass of Eucalyptus pellita EO

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0360	9	0.0040	79.05	< 0.0001	<i>significant</i>
A-Time of extraction	0.0136	1	0.0136	269.33	< 0.0001	
B-Speed of agitation	0.0004	1	0.0004	8.90	0.0204	
C-Ratio solvent: leaves	0.0078	1	0.0078	154.57	< 0.0001	
AB	0.0072	1	0.0072	142.95	< 0.0001	
AC	0.0009	1	0.0009	17.81	0.0039	
BC	0.0056	1	0.0056	111.29	< 0.0001	
A ²	0.0000	1	0.0000	0.08006	0.4006	
B ²	1.516E-06	1	1.516E-06	0.0300	0.8674	
C ²	0.0003	1	0.0003	5.47	0.0520	
Residual	0.0004	7	0.0001			
Lack of Fit	0.0001	3	0.0000	0.7284	0.5863	<i>not significant</i>
Pure Error	0.0002	4	0.0001			
Cor Total	0.0360	16	0.0040			

Table 4. ANOVA quadratic model for the yield of Eucalyptus pellita EO

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0160	9	0.0018	126.02	< 0.0001	<i>significant</i>
A-Time of extraction	0.0062	1	0.0062	441.98	< 0.0001	
B-Speed of agitation	0.0002	1	0.0002	16.43	0.0048	
C-Ratio solvent: leaves	0.0032	1	0.0032	227.53	< 0.0001	
AB	0.0034	1	0.0034	243.33	< 0.0001	
AC	0.0003	1	0.0003	20.55	0.0027	
BC	0.0025	1	0.0025	177.76	< 0.0001	
A ²	0.0001	1	0.0000	2.14	0.1867	
B ²	5.813E-06	1	5.813E-06	0.4133	0.5408	
C ²	0.0000	1	0.0000	3.51	0.1031	
Residual	0.0001	7	0.0000			
Lack of Fit	0.0000	3	0.0000	0.8115	0.5502	<i>not significant</i>
Pure Error	0.0001	4	0.0000			
Cor Total	0.0161	16				

Table 5 shows that the extraction models demonstrate a strong correlation between the observed and predicted values. The coefficient of variance (CV) was used to assess the model's acceptability. Higher repeatability is shown by a smaller CV (less than 10), which indicates that the data points are less dispersed than the mean [21]. This confirms the model's reliability. The coefficient of determination (R^2) was used to assess the model's goodness-of-fit. R^2 measures the proportion of the response variable's total variance explained by the model. R^2 value approaching 1.0 indicates a strong correlation between the predicted and experimental outcomes, substantiating the model reliability [18, 32]. All responses in this investigation had R^2 values more than 0.99, indicating that almost all the observed variability is explained by the regression model.

Table 5. Fit statistics for the mass and yield of Eucalyptus pellita EO

Fit Statistics	Response 1 (Mass of EO)	Response 2 (Yield of EO)
Std. Dev.	0.0071	0.0038
Mean	0.5544	0.3688
CV %	1.28	1.02
R^2	0.9903	0.9939
Adjusted R^2	0.9777	0.9860
Predicted R^2	0.9351	0.9569
Adeq. Precision	31.4069	40.5469

Additionally, the model's predictive reliability is supported, and overfitting is prevented by the agreement between adjusted R^2 and predicted R^2 , with differences of less than 0.2. A slight difference in these measurements points to a well-balanced model that preserves general ability without excessive complexity. Based on the determined ideal factor levels and obtained regression coefficients, the validation procedure demonstrates the model's applicability for precise prediction and optimization of essential oil extraction from Eucalyptus leaves. Furthermore, for every response, the signal-to-noise ratio (a measure of adequate precision) was much higher than 4. This shows that the model signal is substantially stronger than the noise, enabling it to anticipate responses under various experimental conditions and navigate the design space with reliability. Table 5 shows that CV, R^2 , Adjusted R^2 , Predicted R^2 , and Adequate Precision meet model evaluation requirements for both mass and yield of EO. Then, it can be forwarded to the optimization stage [18].

In the context of RSM, the model's standard deviation is the residual standard error, also known as the root mean square error (RMSE). It represents the typical size of a prediction error. A smaller standard deviation indicated that the model has high precision. Based on Table 5, the standard deviation for mass and yield of EO were 0.0071 and 0.0038.

Using paired data from 17 experiments and correlation analyses, the ANOVA Quadratic Model presents mathematical equations for mass and yield in terms of actual factors. The resulting response equations derived from the model are presented in Equations 2 and 3 for the coded equation and Equations 4 and 5 for the actual equation. Y_a represents the predicted response for mass, and Y_b represents the predicted response for the yield of EO. For both mass and yield of EO, the quadratic term (A^2 , B^2 , C^2) shows no significance to the responses. So, the coefficient and term in the mathematical Equations 2 to 5 can be omitted.

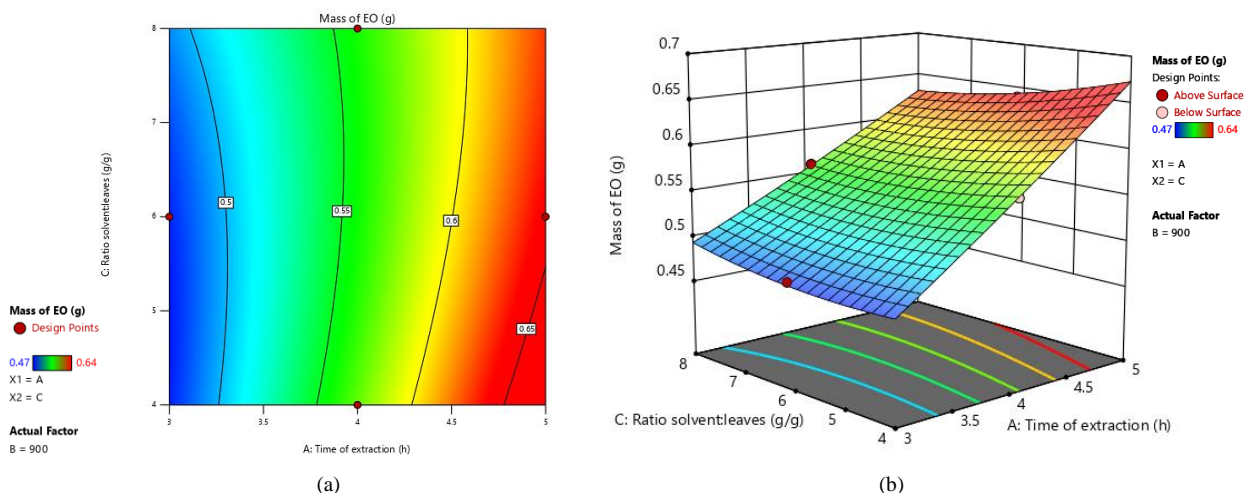
$$Y_a = 0.5488 + 0.0412A + 0.0075B + 0.0313C + 0.0425AB - 0.0150AC - 0.0375BC \quad (2)$$

$$Y_b = 0.3654 + 0.0279A + 0.0054B + 0.0200C + 0.0293AB - 0.0085AC - 0.0250BC \quad (3)$$

$$Y_a = 0.334950 - 0.023550 \times (\text{extraction time}) - 0.000175 \times (\text{agitation speed}) + 0.058825 \times (\text{solvent} - \text{to} - \text{leaves ratio}) + 0.0000142 \times (\text{extraction time}) \times (\text{agitation speed}) - 0.007500 \times (\text{extraction time}) \times (\text{solvent} - \text{to} - \text{leaves ratio}) - 0.000062 \times (\text{agitation speed}) \times (\text{solvent} - \text{to} - \text{leaves ratio}) \quad (4)$$

$$Y_b = 0.233475 - 0.026525 \times (\text{extraction time}) - 0.000138 \times (\text{agitation speed}) + 0.041725 \times (\text{solvent} - \text{to} - \text{leaves ratio}) + 0.000098 \times (\text{extraction time}) \times (\text{agitation speed}) - 0.004250 \times (\text{extraction time}) \times (\text{solvent} - \text{to} - \text{leaves ratio}) - 0.000042 \times (\text{agitation speed}) \times (\text{solvent} - \text{to} - \text{leaves ratio}) \quad (5)$$

The response surface is used to interpret the interaction among variables and the optimum level of each variable for maximum response [20]. Figures 2 and 3 show the two-dimensional (2D) and three-dimensional (3D) contours for the surface of various variable correlations. The 3D curvature of the surface response indicates that the mathematical equation model is nonlinear. This 3D visualization (Figures 2-b, 2-d, 2-f and 3-b, 3-d, 3-f) is in accordance with ANOVA results, indicating that the mathematical model is quadratic. Based on Figures 2-a and 2-b, variable A is the dominant factor compared to C. Figures 2-c and 2-d shows a relative relationship between extraction time and stirring speed with an increase in the amount of EO extracted. Figures 2-e and 2-f shows a positive correlation between variables B and C, indicating that elevating the solvent-biomass ratio while accelerating stirring can increase the amount of EO produced. The yield also shows the same profile as the mass of EO (Figure 3). The most dominant factor in the mass and yield of EO is extraction time (A).



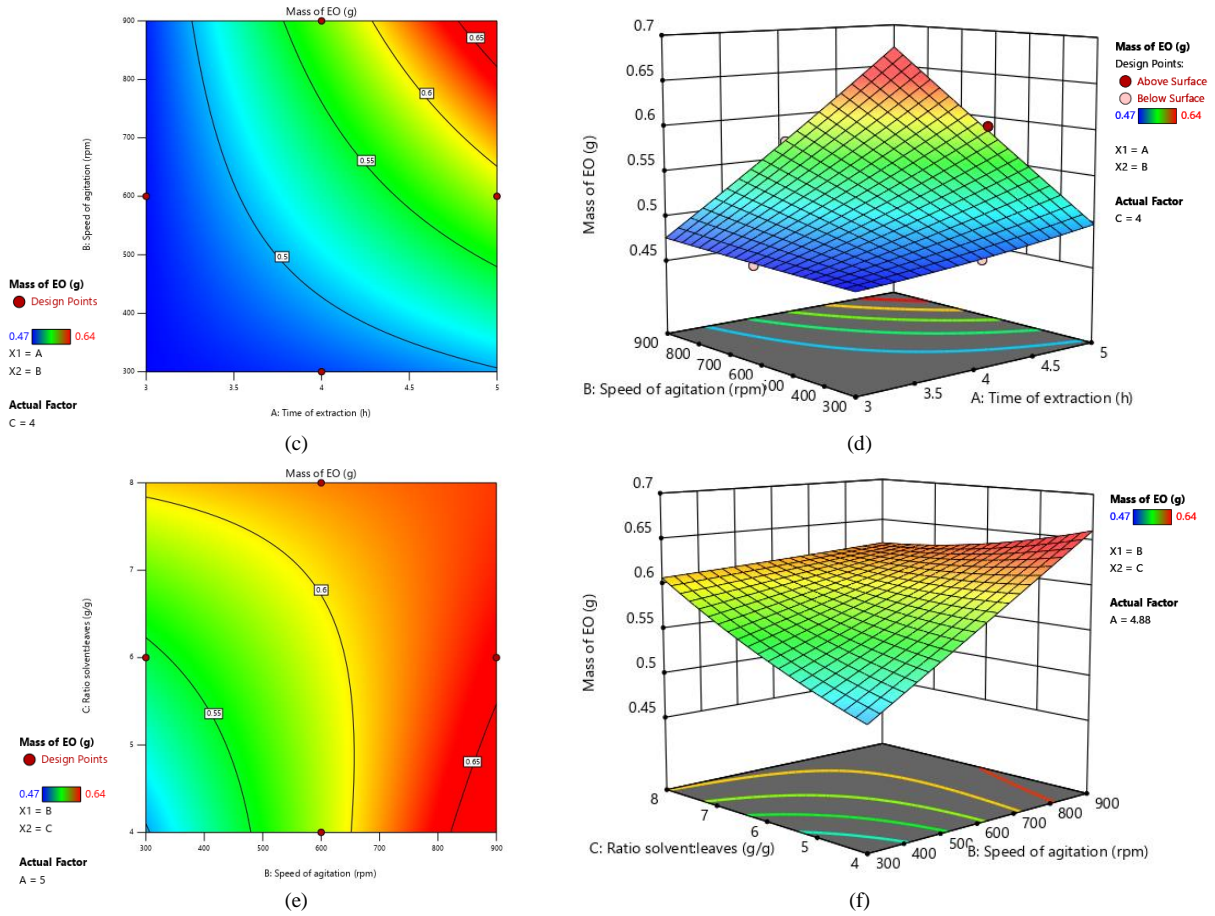
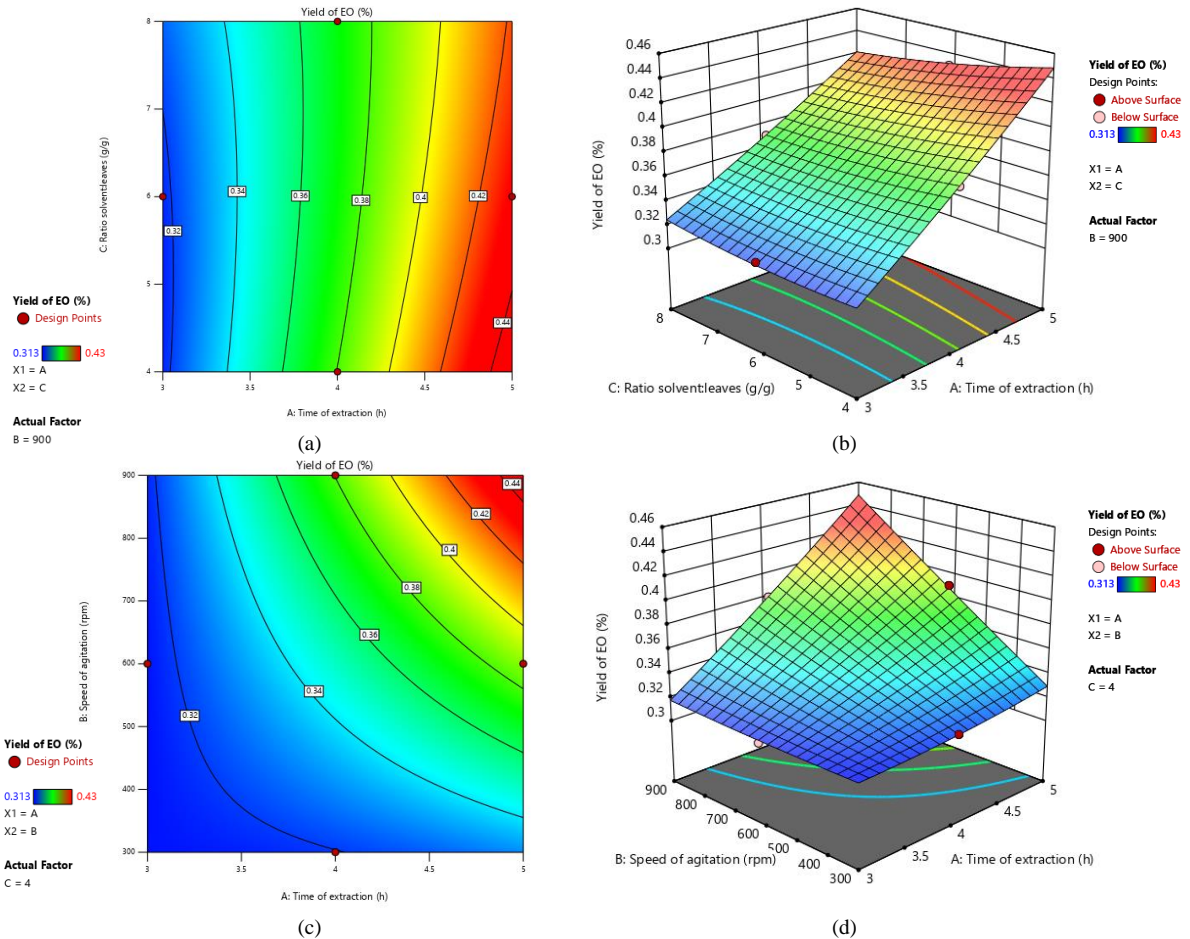


Figure 2. 2D and 3D visualizations for mass of EO generated from HD methods; (a) 2D AC interaction, (b) 3D AC interaction, (c) 2D AB interaction, (d) 3D AB interaction, (e) 2D BC interaction, (f) 3D BC interaction



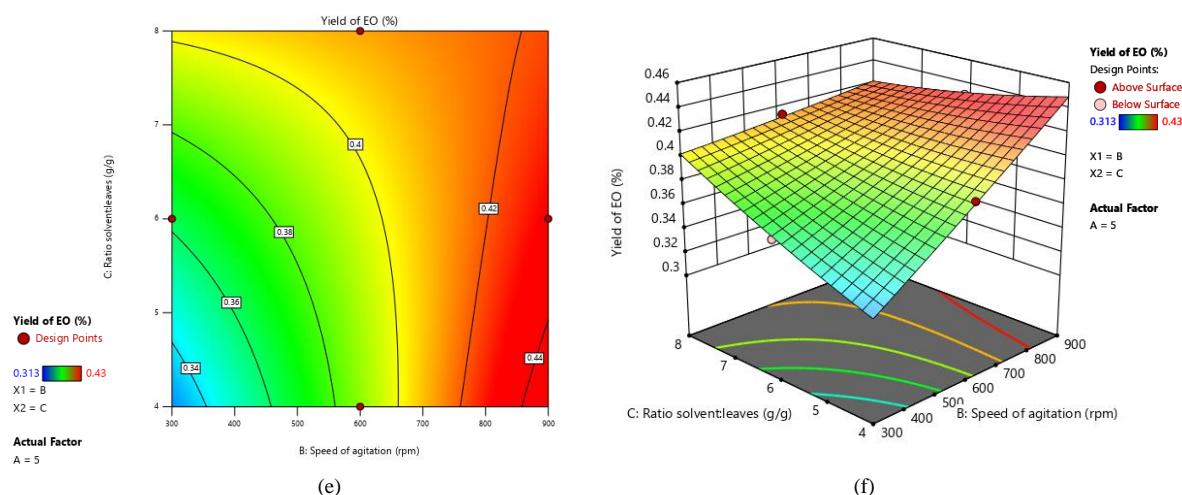


Figure 3. 2D and 3D visualizations for yield of EO generated from HD methods; (a) 2D AC interaction, (b) 3D AC interaction, (c) 2D AB interaction, (d) 3D AB interaction, (e) 2D BC interaction, (f) 3D BC interaction

The colors on the surface response describe the interaction between the levels of the variables used. The lowest level is shown in blue, the middle level in green, and the highest level in red. Significant interactions between variables result in the maximum response value, depicted in red. On the other hand, for interactions with minimum response, it is depicted in blue. This color depiction also corresponds to the same profiles shown in Figures 3 and 4. For example, the results show that the increase in extraction time and agitation speed produces the maximum EO mass and yield, as depicted in red in the upper right corner of Figure 2-c and 2-d and Figures 3-c and 3-d.

Through further analysis, the specification of the theoretical optimal variables and the experimental response (confirmation of laboratory results) are shown in Tables 6 and 7. According to Table 6, predicted optimal conditions for EO extracted from *Eucalyptus pellita* are at 4.97 h of extraction time, 873.6 rpm of agitation speed, and 5.4 of solvent-to-leaves mass ratio. The experimental confirmation of these optimal conditions shows that the average responses for mass and yield of EO were 0.635 g and 0.4235%, respectively. Table 7 presents confirmation of optimized variables between the predicted and actual values, where Std. Dev. refers to standard deviation, n refers to the number of experimental data points, and SE. Pred. refers to standard error prediction, and PI refers to prediction interval. RSM quantifies the uncertainty around a predicted response at specific factor settings, using a 95% PI. If there are many repetitions of the experiments at the same factor settings, 95% of the observed response values will fall between PI low and PI high. That means the confidence of this prediction model is 95%. Table 7 shows that the predicted mass ranges from 0.62 to 0.66 grams, and the yield ranges from 0.42 to 0.44%. The response data for the experiments are between PI low and PI high. Table 7 also shows the standard errors of 0.0076 (or 0.76%) for the mass and 0.0040 (or 0.4%) for the yield of EO.

Table 6. Optimized conditions for the HD process

Response Data	A (h)	B (rpm)	C (g/g)	Experimental Mass of EO (g)	Experimental Yield of EO (%)
1	4.97	873.6	5.4	0.63	0.420
2				0.64	0.427
Average				0.635	0.4235

Table 7. Confirmation of optimized conditions for the HD process

Analysis	Predicted Mean	Predicted Median	Std. Dev.	n	SE Pred.	95% PI low	Data Mean	95% PI high
Mass of EO	0.642366	0.642366	0.0071	2	0.0076	0.624376	0.635	0.660355
Yield of EO	0.430132	0.430132	0.0038	2	0.0040	0.420642	0.4235	0.439622

The optimal value of the selected factor and response corresponds to the highest desirability value, typically above 0.9. The criteria can be adjusted as needed to achieve satisfactory desirability. The predicted response corresponds to the experimental response value, indicating that the desirability function is an efficient method to optimize the extraction method. The optimization of *Eucalyptus pellita* EO using RSM and HD can be effectively evaluated using desirability. This function integrates multiple responses, including yield, mass, and concentrations of bioactive compounds such as 1,8-cineole, into a single numerical value ranging from 0 to 1. Figure 4 presents that a desirability value of this research is 1 indicates very optimal extraction conditions. Based on laboratory experiments and data analysis, the theoretical mathematical model is feasible for optimized HD operating conditions in the extraction of *Eucalyptus pellita* EO. This integrated approach ensures a balance between quality and quantity, making HD more effective and sustainable for industrial-scale EO production of *Eucalyptus pellita*.

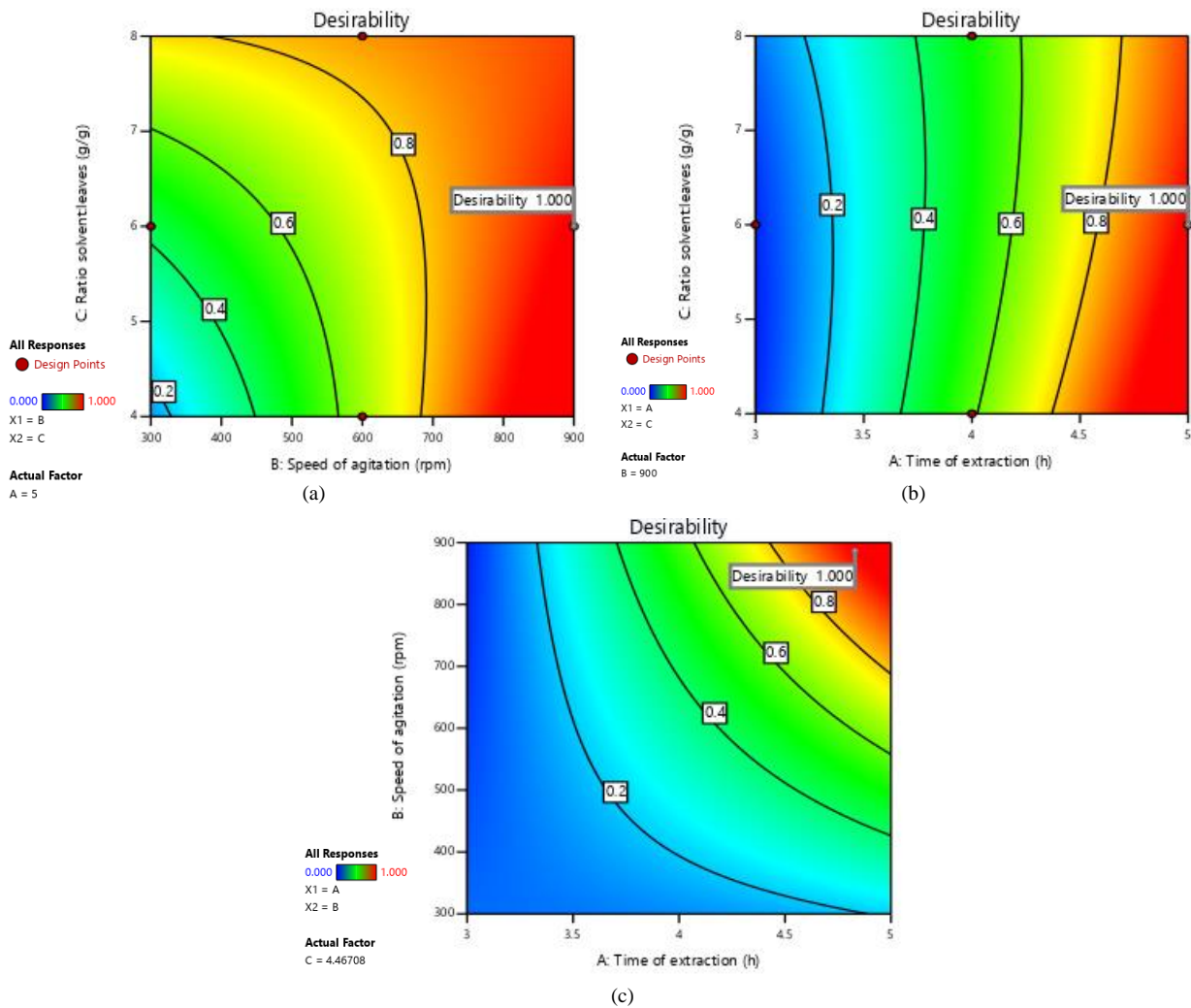


Figure 4. Desirability visualization for EO generated from the HD method; (a) BC interaction, (b) AC interaction, (c) AB interaction

A related study applied RSM combined with desirability analysis to optimize EO recovery from *Eucalyptus globulus* leaves through HD [18]. The results showed a maximum desirability value of 0.652 under optimal conditions, including leaf size 0.02 m, temperature 97.76 °C, solid-to-solvent ratio 0.61, and extraction time 206 minutes, which resulted in an EO yield of 0.70%, with 1.8-cineole at 63.98% and α -pinene at 20.81% [18]. This suggests that desirability does not necessarily maximize each response individually but rather balances multiple parameters to achieve overall efficiency in the extraction process.

Based on the F- and p-values in Tables 3 and 5, the order of factors that significantly affect the response (mass and yield of EO) is A, C, AB, BC, AC, and B. The agitation speed factor (B) has an effect, but its magnitude is the smallest among the factors. This is consistent with the study by Lainez-Cerón et al. [27], which examined the solid-liquid ratio and agitation speed in HD *Eucalyptus urograndis* using a 2^3 full factorial design. Their statistical analysis revealed that neither agitation speed nor its interactions had a significant effect on the yield, which depended solely on the solid-liquid ratio used [27].

A study of RSM-BBD for *Eucalyptus tereticornis* EO from Nigeria reported that solute/solvent ratio, time, and steam rate were factors that affected the EO produced by steam distillation [20]. Based on ANOVA, the solute/solvent ratio did not significantly affect the yield of EO [20]. The optimum condition at 105 minutes of extraction and a steam rate of 0.032 kg/h produced 2.05% yield of EO [20].

Sareriya et al. (2024) reported RSM-CCD for *Eucalyptus hybrid* EO by using steam distillation. The optimum conditions were leaf size: 0.02 m, temperature: 97.76 °C, solid to solvent ratio: 0.61, and time: 206 min, yielding 0.70% oil [18]. They reported that the leaf size has a significant influence on the yield of EO, generally increasing to the optimal point [18]. Abed & Naife (2018) showed that the time, temperature, agitation speed, ratio of solvent to biomass, and particle size affect the amount of EO produced [30]. In their study, the five factors increased to a certain extent in line with the rise in the amount of EO obtained [30]. This is due to an increase in concentration gradient between the liquid and solid phases, which promotes more efficient mass transfer. However, an excessive or low amount of the factors may not significantly affect the extraction process, because of the equilibrium state.

Eucalyptus globulus from Pakistan was studied by using superheated steam extraction to enhance the EO produced. They reported RSM-CCD with three factors: temperature, time, and steam rate. The equipment used was a superheated steam extractor from PAMICO Technology, Pakistan. The optimal conditions were at 140°C for 40 minutes and a steam rate of 60 ml/minute, which produced a 1.73% yield of EO, exceeding that obtained with conventional HD and SD [21].

A study on *Eucalyptus camaldulensis* was conducted using RSM, with three methods applied: FFD, CCD, and BBD. They observed vapor volume, distillate flow rate, and particle size as the factors that affect the yield of EO. A maximum yield of 0.7 ml of oil was produced under BBD-optimum conditions, which included a hydrodistillation duration of 6 h, a distillate flow rate of 1 to 10 ml/min, a vapor volume of 1348.33 ml, and a pulverization degree of 0.375 mm [17].

Eucalyptus pellita from Cibunar Hamlet, Cisarua Village, Samarang District, Garut Regency, West Java, Indonesia, used as raw material, produced a 0.34% yield of EO extracted using HD under conditions of 5 hours with a solid-to-leaf ratio of 1:7 [33]. Another HD research by using *Eucalyptus pellita* from India as raw materials produced a 0.26-0.44% yield of EO [34]. A study of *Eucalyptus pellita* from East Kalimantan, Indonesia, produced EO by using the steam distillation method, with an amount of 0.86% yield of EO produced for a 4h extraction [35], and 0.44% yield of EO for 5h extraction [36]. The present study showed better results compared to [33], in line with [34, 36], and lower than [35]. This reveals how the composition and yield of EO are influenced not only by parameters of extraction conditions, but also by environmental factors such as soil composition, climate, and altitude. A summary of the comparison between this study and previous references is shown in Table 8.

Table 8. Comparison of optimal conditions for EO extraction

Raw Material	Extraction Time (minutes)	Agitation Speed (rpm)	Ratio biomass: solvent (g/ml)	Optimization Method	Yield (%)	Reference
<i>E. urograndis</i>	90	400	1:05	2 ³ FFD	1.19	Lainez-Cerón et al. [27]
<i>E. tereticornis</i>	105	-	-	BBD	2.05	Galadima et al. [20]
<i>E. hybrid</i>	206	-	0.61:1	CCD	0.7	Sareriya et al. [18]
<i>E. camaldulensis</i>	360	-	1:10	BBD	0.7*	Labri et al. [17]
<i>E. globulus</i>	40	-	01:02.4	CCD	1.73	Ayub et al. [21]
<i>E. pellita</i>	298	873.6	01:05.4	BBD	0.4235	Present study

* ml

4- Conclusion

This study successfully optimized the HD of *Eucalyptus pellita* EO using RSM coupled with a BBD. The statistical analysis validated the high reliability and predictive capability of the generated quadratic models for both mass and yield EO. The model fitted to the experimental data, as evidenced by a high coefficient of determination ($R^2 > 0.99$), a significant model ($p < 0.05$), and a non-significant lack-of-fit ($p\text{-value} > 0.05$). The model accurately captures the relationship between the process variables and the responses.

The ANOVA p-value showed that all three linear factors (time, agitation speed, and solvent-to-leaf mass ratio) and their interaction significantly affect extraction efficiency. However, the p-values for the quadratic terms (A^2 , B^2 , C^2) were greater than 0.05, indicating non-significance. Their inclusion in the model provided a complete description of the response surfaces. The model for both EO mass and yield also demonstrated strong statistical adequacy, including the coefficient of determination (R^2), adjusted R^2 , predicted R^2 , and adequate precision. These values indicated a significant signal and reliable predictive power within the design.

The numerical optimum extraction conditions were identified at an extraction time of 4.96 h, an agitation speed of 873.6 rpm, and a leaf-to-solvent mass ratio of 1:5.4. This condition predicted 0.62-0.66 g of mass and 0.42-0.44% of yield EO. Under these optimized conditions, the experimental validation yielded EO with an average mass of 0.635 g and a yield of 0.4235%, demonstrating excellent agreement with the model-predicted values. This close alignment highlights the model's practical utility. This study not only offers a precise set of optimal parameters to maximize EO recovery from *Eucalyptus pellita* but also provides a scientific validation of RSM-BBD as a potent and effective tool for modeling and optimizing complex extraction processes. The findings underscore a foundational scientific basis for scaling up and enhancing the industrial valorization of *Eucalyptus pellita* EO, thereby promoting a more efficient and predictable production process.

5- Declarations

5-1- Author Contributions

Conceptualization, S.A. and S.M.; methodology, L.C.; software, L.C.; validation, L.C., S.A., and P.L.H.; formal analysis, S.A.; investigation, L.C.; resources, S.; data curation, L.C. and P.L.H.; writing—original draft preparation, L.C.; writing—review and editing, L.C., S.A., and P.L.H.; visualization, L.C. and P.L.H.; supervision, S. A., P.L.H., and S.M.; project administration, L.C.; funding acquisition, S. All authors have read and agreed to the published version of the manuscript.

5-2-Data Availability Statement

The data presented in this study are available on request from the corresponding author.

5-3-Funding and Acknowledgments

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5-4-Institutional Review Board Statement

Not applicable.

5-5-Informed Consent Statement

Not applicable.

5-6-Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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