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Optimization of Black Orchid (*Coelogyne pandurata* Lindl.) Extraction Using Simplex-Centroid Design for Total Phenolics, Flavonoids, and Antioxidants

Optimasi Ekstraksi Anggrek Hitam (*Coelogyne pandurata* Lindl.) dengan Simplex-Centroid Design untuk Fenolik, Flavonoid, dan Antioksidan

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ABSTRACT

Coelogyne pandurata Lindl., a black orchid endemic to Kalimantan, contains valuable antioxidant compounds. This study optimized its extraction using a Simplex-Centroid Mixture Design with ethanol, methanol, and acetic acid as solvents. Responses evaluated were total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity (DPPH). The highest TPC was obtained with 100% ethanol (2.52 mg GAE/g), TFC with equal parts of all solvents (6.41 mg QE/g), and antioxidant activity with 100% methanol (10.33 µmol TE/g). Ethanol yielded the optimal extraction condition, with verification values of 2.27 mg GAE/g (TPC), 3.42 mg QE/g (TFC), and 10.22 µmol TE/g (DPPH), and a desirability of 0.739. Although regression models were not statistically significant ($p > 0.05$), the results provide preliminary insights into solvent efficiency for extracting bioactive compounds. Further studies with expanded experimental designs and validation are required to enhance model accuracy and extraction reliability.

Kata kunci:
Anggrek hitam
Desain ekstraksi
Senyawa antioksidan

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ABSTRAK

Anggrek hitam (*Coelogyne pandurata* Lindl.) merupakan tanaman endemik Kalimantan yang kaya senyawa antioksidan. Penelitian ini mengoptimasi ekstraksi menggunakan desain Simplex-Centroid dengan pelarut etanol, metanol, dan asam asetat. Respon yang dianalisis meliputi kandungan fenolik total (TPC), flavonoid total (TFC), dan kapasitas antioksidan (DPPH). Nilai TPC tertinggi diperoleh dengan 100% etanol (2,52 mg GAE/g), TFC tertinggi pada campuran 33,3% tiap pelarut (6,41 mg QE/g), dan kapasitas antioksidan tertinggi dengan 100% metanol (10,33 µmol TE/g). Etanol menghasilkan kondisi optimum dengan nilai verifikasi sebesar 2,27 mg GAE/g (TPC), 3,42 mg QE/g (TFC), dan 10,22 µmol TE/g (DPPH), serta desirability 0,739. Meskipun model regresi tidak signifikan ($p > 0{,}05$), hasil ini memberikan informasi awal tentang efektivitas pelarut dalam mengekstraksi senyawa bioaktif. Studi lanjutan dengan variasi pelarut dan validasi laboratorium diperlukan untuk meningkatkan keakuratan model dan efisiensi ekstraksi.

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1. INTRODUCTION

The black orchid (*Coelogyne pandurata* Lindl.) is an endemic species of Kalimantan, recognized for its potential as a natural source of bioactive secondary metabolites, particularly phenolic and flavonoid compounds with antioxidant properties (Heriansyah et al., 2025a). These metabolites are widely valued in pharmaceutical, nutraceutical, and functional food industries due to their ability to scavenge free radicals and mitigate oxidative stress, thereby contributing to disease prevention and health maintenance (Soussi et al., 2022).

The successful utilization of plant-derived antioxidants largely depends on the effectiveness of the extraction process. Among various factors, solvent selection plays a critical role in determining extraction efficiency, as differences in polarity, solubility, and interaction with plant matrices influence compound recovery (El Mannoubi, 2023). Ethanol and methanol are commonly used polar solvents for phenolic and flavonoid extraction, while acetic acid may enhance the release of complex or bound phytochemicals within plant tissues. Previous studies on *C. pandurata* have demonstrated variations in phenolic and flavonoid content depending on plant part and environmental conditions (Heriansyah et al., 2025b), underscoring the need for a systematic extraction strategy. The application of mixture design models offers a promising approach to optimize solvent combinations for maximizing the yield of multiple bioactive compounds.

The Simplex-Centroid Mixture Design (SCD) is a statistical method specifically designed to evaluate the effects of mixture proportions rather than absolute quantities. It facilitates the identification of optimal solvent compositions by modeling interactions among solvents and predicting multiple response variables (Navarro-Cortez et al., 2023). SCD has been successfully applied in phytochemical extractions due to its efficiency and suitability for response surface analysis (Jdaini et al., 2023).

Therefore, this study aimed to optimize the composition of ethanol, methanol, and acetic acid for the extraction of phenolics, flavonoids, and antioxidant compounds from *C. pandurata* using the SCD approach. The outcomes are expected to provide insights into solvent-matrix interactions and support the development of efficient extraction protocols for black orchid-derived bioactives.

2. METHODS

2.1. Materials and Equipment

The plant materials included leaves, flowers, and pseudobulbs of *C. pandurata*. Solvents and reagents used were of analytical grade, including ethanol, methanol, glacial acetic acid, Folin–Ciocalteu reagent, aluminum chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), DPPH (2,2-diphenyl-1-picrylhydrazyl), and standard compounds (gallic acid, quercetin, and Trolox), all obtained from Merck KGaA (Germany).

Laboratory equipment included glassware (Erlenmeyer flasks, beakers, volumetric flasks, pipettes), an analytical balance (Ohaus, USA), blender (Philips, Netherlands), drying oven (Memmert, Germany), microwave-assisted extractor, refrigerator, nanospectrophotometer (SPECTROstar Nano, BMG LABTECH, Germany), 96-well microplates, micropipettes (Eppendorf, Germany), shaker, water bath, and a pH meter (Hanna Instruments, USA).

2.2. Experimental Design

Solvent optimization was conducted using the Simplex-Centroid Mixture Design (SCD) with Design-Expert® version 13.0 (Stat-Ease Inc., USA). The independent variables were the proportions of ethanol, methanol, and acetic acid, while the responses measured included total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity (DPPH). The design matrix is presented in **Table 1**.

2.3. Sample Preparation and Extraction Procedure

Leaves, flowers, and pseudobulbs were combined into a composite sample, oven-dried at 50 °C for 72 hours, ground, and passed through a 60-mesh sieve. A total of 4 g of the dried sample was extracted using various solvent mixtures according to the SCD matrix. Microwave-assisted extraction (MAE) was carried out at 135 W for 3 minutes, following Nurcholis et al. (2022) with modifications to the solvent composition and sample mass. The extracts were filtered, and the volume was adjusted to match the initial solvent volume.

Table 1. Experimental design matrix based on Simplex-Centroid Mixture Design

Run	Ethanol (%)	Methanol (%)	Acetic acid (%)
1	0.00	0.00	100.00
2	0.00	100.00	0.00
3	50.00	0.00	50.00
4	50.00	0.00	50.00
5	33.33	33.33	33.33
6	66.67	16.67	16.67
7	0.00	0.00	100.00
8	100.00	0.00	0.00
9	16.67	66.67	16.67
10	0.00	100.00	0.00
11	16.67	16.67	66.67
12	50.00	50.00	0.00
13	0.00	50.00	50.00
14	50.00	50.00	0.00
15	100.00	0.00	0.00
16	100.00	0.00	0.00
17	0.00	50.00	50.00

2.4. Total Phenolic Content (TPC) Determination

TPC was determined using a modified Folin–Ciocalteu method (Nurcholis et al., 2022). Briefly, 20 µL of extract was mixed with 120 µL of 10% (v/v) Folin–Ciocalteu reagent, incubated in the dark for 5 minutes, followed by the addition of 80 µL of 10% (w/v) Na_2CO_3. After 30 minutes of incubation at room temperature, absorbance was measured at 750 nm using a nanospectrophotometer. Gallic acid (20–300 ppm) was used for calibration, and results were expressed as mg gallic acid equivalent (GAE)/g fresh weight (fw).

2.5. Total Flavonoid Content (TFC) Determination

TFC was assessed using the aluminum chloride colorimetric method (Nurcholis et al., 2022), with slight modifications. A mixture containing 10 µL extract, 120 µL distilled water, 10 µL of 10% $AlCl_3$, 10 µL glacial acetic acid, and 50 µL ethanol was incubated at room temperature for 30 minutes in the dark. Absorbance was recorded at 415 nm. Quercetin (0–500 ppm) was used as a standard, and results were expressed as mg quercetin equivalent (QE)/g fw.

2.6. Antioxidant Capacity (DPPH Assay)

The antioxidant capacity was measured using the DPPH radical scavenging method (Arista & Nurcholis, 2024), with minor modifications. A total of 100 µL extract was added to 100 µL of 125 µM DPPH in ethanol, then incubated for 30 minutes in the dark. Absorbance was read at 515 nm. Trolox (0–50 µM) was used as a reference standard, and results were expressed as µmol Trolox equivalent (TE)/g fw.

2.7. Data Analysis

All data were subjected to analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level using IBM SPSS Statistics version 25. Optimization was performed using the desirability function in Design-Expert® 13.0, and the optimal solution was selected based on the highest desirability value.

3. RESULTS AND DISCUSSION

3.1. Extraction Performance Based on Simplex-Centroid Mixture Design

Extraction optimization plays a critical role in maximizing the recovery of target phytochemicals in medicinal plant research (Azahar et al., 2020). In this study, microwave-assisted extraction (MAE) was applied due to its reported superiority over other methods such as ultrasound-assisted extraction (UAE) and continuous shaking extraction (CSE), particularly for polyphenol recovery (Routray & Orsat, 2012; Nurcholis et al., 2022).

As presented in **Table 2**, the highest total phenolic content (TPC) was observed in Run 16 using 100% ethanol, yielding 2.52 mg GAE/g dry weight (DW), followed closely by Run 15 with 2.49 mg GAE/g DW. These results indicate ethanol as the most effective solvent for extracting phenolics from *C. pandurata*. In contrast, the highest total flavonoid content (TFC) was recorded in Run 5 (6.41 mg QE/g DW), which used an equal proportion (33.33%) of ethanol, methanol, and acetic acid. This suggests that moderate polarity combinations enhance flavonoid extraction. The highest antioxidant activity, measured by DPPH, was found in Run 10 using 100% methanol (10.33 µmol TE/g DW), indicating methanol's strong affinity for antioxidant-active compounds.

Table 2. Experimental results of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity (DPPH) obtained from the Simplex-Centroid Mixture Design.

Run	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH (µmol/g DW)
1	0.847538	0.62119	9.23534
2	1.85511	4.01167	10.3037
3	2.35701	4.18786	10.1356
4	2.02367	5.80452	10.0564
5	2.2358	6.41405	10.1257
6	1.14678	1.30214	9,819
7	2.35133	3.88786	10.2741
8	2.40246	3.52595	10.2345
9	2.00284	3.25929	10.1257
10	2.19034	4.81405	10.3334
11	0.940341	0.297381	8.70115
12	2.01231	2.94262	9.93771
13	2.4233	2.69976	10.1751
14	2.1411	4.82357	10.2741
15	2.49148	3.23786	10.2147
16	2.5161	3.24738	10.2543
17	2.4214	3.1069	10.2444

Notes: TPC is expressed as mg gallic acid equivalent (GAE)/g dry weight (DW), TFC as mg quercetin equivalent (QE)/g DW, and antioxidant capacity as µmol Trolox equivalent (TE)/g DW.

3.2. Regression Model and Statistical Evaluation

Response surface modeling was conducted to evaluate the relationships between solvent proportions and the responses. As shown in **Table 3**, TPC followed a linear model, DPPH fit a special quadratic model, while TFC yielded no suitable model. Statistical evaluation using ANOVA (**Table 4**) revealed that none of the models were statistically significant ($p > 0.05$), and the R^2 values were below acceptable thresholds, indicating weak model predictability.

The limited significance of these models can be attributed to several factors, including the complexity of the plant matrix, variability in compound stability, and narrow solvent composition ranges. The weak correlation is further illustrated by the flat response surfaces in **Figure 1**, which show minimal changes across different solvent combinations.

The lack of significant regression models aligns with findings from similar mixture design studies (Navarro-Cortez et al., 2023; Cavalcanti et al., 2021). Variability in plant matrices, degradation of secondary metabolites during extraction, and overlapping solvent effects often result in low response differentiation. In particular, solvent interactions may not always produce synergistic effects, and mixtures can yield similar extractability as single solvents (Jdaini et al., 2023). Furthermore, extraction parameters such as temperature, particle size, and exposure time, although partially controlled, may have contributed to variation in the results, potentially acting as confounding factors in the modeling process.

Table 3. Regression equations generated by the Simplex-Centroid Mixture Design for TPC, TFC, and DPPH responses.

Response	Model	Equality Regression
TPC	Linear	$Y = 2.27A + 2.11B + 1.65C$
TFC	-	$Y = 3.42$
DPPH	Special Quadratic	$Y = 10.22A + 10.29B + 9.73C - 0.8060AB + 0.2821AC + 0.5867BC + 15.07A^2BC + 41.38AB^2C - 91.81ABC^2$

Notes: A, B, and C represent the proportions of ethanol, methanol, and acetic acid, respectively.

Table 4. Analysis of variance (ANOVA) results for regression models of TPC, TFC, and antioxidant capacity (DPPH)

Variables	TPC	TFC	DPPH
	Linear model	Nothing fits	Special Quadratic
F	1.12	Undefined	2.22
p	0.3537	Undefined	0.1632
R^2	0.1380	0.00	0.6107
Adjusted R^2	0.0148	0.00	0.2213

Notes: R^2 indicates the coefficient of determination, adjusted R^2 accounts for model complexity, and p-values > 0.05 indicate non-significant models.

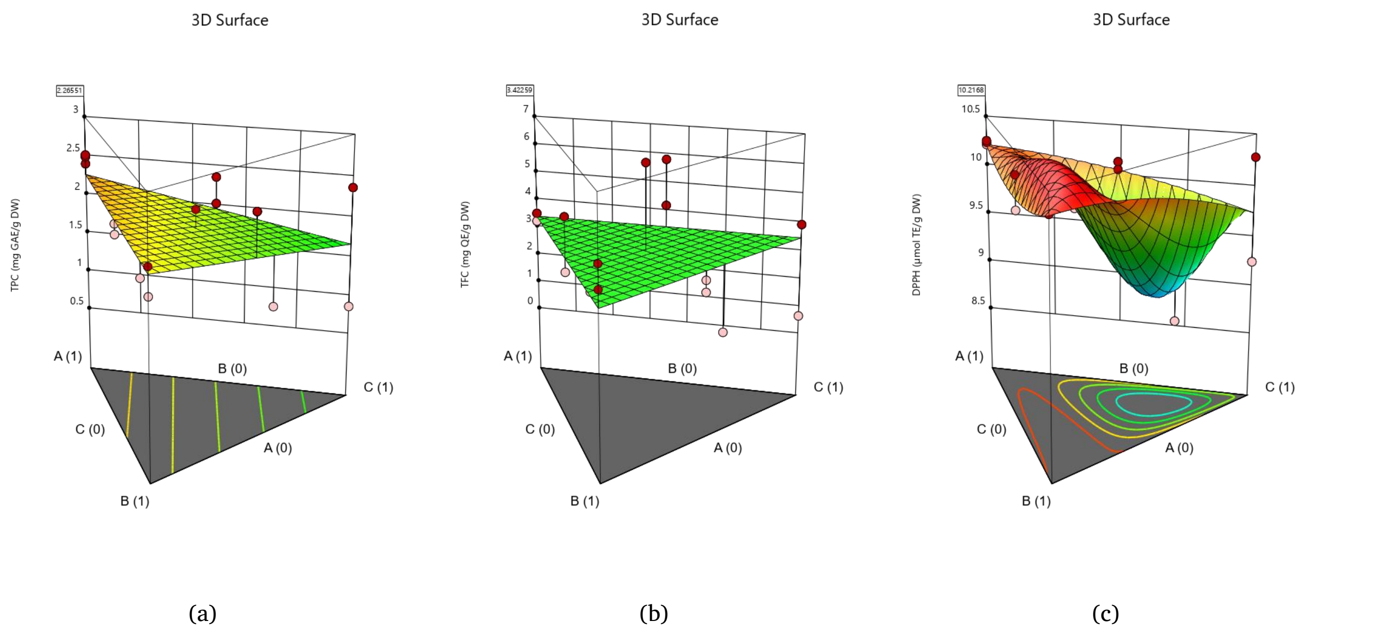

Figure 1. Three-dimensional response surface plots showing the effects of solvent proportions on (a) total phenolic content (TPC), (b) total flavonoid content (TFC), and (c) antioxidant capacity (DPPH). Relatively flat surfaces indicate weak relationships between solvent composition and response variables within the tested range.

3.3. Solvent Optimization and Verification

Despite the limitations, the SCD analysis yielded a solvent combination with the highest desirability score. As shown in **Table 5**, 100% ethanol produced the optimal condition with a desirability value of 0.739. The model predicted TPC, TFC, and DPPH values of 2.27 mg GAE/g, 3.42 mg QE/g, and 10.22 µmol TE/g, respectively. These values were closely aligned with experimental results, validating ethanol's practical effectiveness as an extraction solvent for phenolic-rich fractions from *C. pandurata*. The 3D desirability surface (**Figure 2**) also illustrated ethanol's consistent contribution to maximizing the overall response, despite the overall statistical insignificance of the model.

3.4. Practical Implications and Future Direction

Although statistical validation was limited, the trends observed are practically relevant. Ethanol demonstrated the most consistent performance for phenolic extraction, while methanol showed superior antioxidant activity. Mixed solvents may still offer value for broader-spectrum recovery of flavonoids.

To improve model robustness and extraction efficiency, future studies should incorporate expanded solvent ratio ranges, increased experimental runs and replications, and integration of other extraction parameters such as time, temperature, and particle size. Applying alternative modelling approaches (e.g., D-optimal or Box–Behnken design) and validating predicted solutions through laboratory trials will also be essential for standardizing black orchid extract formulations for functional applications.

Table 5. Optimization solutions generated by the Simplex-Centroid Mixture Design based on desirability function analysis.

Number	*Ethanol*	*Methanol*	*Acetic acid*	TPC	TFC	DPPH	Desirability
1	100	0	0	2,266	3,423	10,217	0.739
2	53.2	40.1	6.8	2.16	3,423	10,333	0.738
3	0	100	0	2.16	3,423	10,292	0.721

Notes: The optimal solution was selected according to the highest desirability value for simultaneous optimization of TPC, TFC, and DPPH.

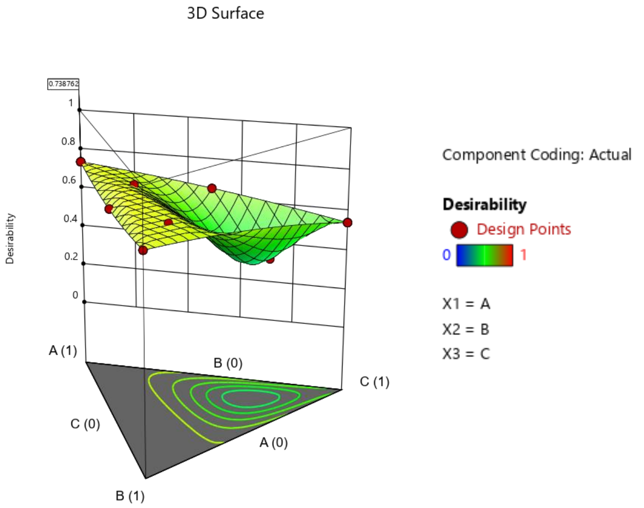

Figure 2. Three-dimensional desirability surface plot illustrating the combined optimization of TPC, TFC, and antioxidant capacity (DPPH) based on solvent composition. Higher desirability values indicate more favorable extraction conditions.

4. CONCLUSION

This study showed that ethanol, methanol, and acetic acid affected the extraction efficiency of *C. pandurata*, with ethanol yielding the highest total phenolic content (2.52 mg GAE/g DW), methanol providing the highest antioxidant capacity (10.33 µmol TE/g DW), and mixed solvents (33.3% each) resulting in the highest flavonoid content (6.41 mg QE/g DW). Although regression models were not statistically significant ($p > 0.05$), ethanol was identified as the optimal solvent with a desirability of 0.739 and verification values of 2.27 mg GAE/g (TPC), 3.42 mg QE/g (TFC), and 10.22 µmol TE/g (DPPH). These findings offer practical insights for solvent selection and highlight the need for extended experimental designs and model validation in future optimization studies.

AUTHOR CONTRIBUTIONS

Conceptualization: P.H., W.N.; Methodology: P.H.; Validation: P.H., W.N., N.L.; Formal analysis: N.L.; Investigation: P.H.; Resources: W.N.; Data curation: P.H., N.L.; Writing – original draft: N.L.; Writing – review & editing: P.H., W.N.; Visualization: N.L.; Supervision: W.N.; Project administration: N.L. All authors have read and approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, and readability of the text. Following the use of this tool, the authors critically reviewed, edited, and verified the entire manuscript to ensure that it accurately reflects their own scientific interpretations and conclusions. The authors take full responsibility for the originality, accuracy, and integrity of the published work.

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