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Antioxidant activity and secondary metabolite profile of protocorm-like bodies (PLBs) of *Dendrobium crumenatum* and *Dendrobium liniale*

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Abstract

Background: The Dendrobium orchid genus is recognized as a source of secondary metabolites with potential therapeutic applications. Sustainable biomass availability can be addressed through the induction of protocorm-like bodies (PLBs). **Objective**: This study aims to observe the growth of PLBs, screen secondary metabolites, and compare the antioxidant activity between PLBs extracts and plant extracts. **Methods:** The growth of PLBs *Dendrobium crumenatum* and *Dendrobium liniale* was observed until day 60. Secondary metabolite screening was carried out qualitatively to identify the presence of alkaloids, phenolics, tannins, flavonoids, saponins, steroids, and terpenoids. Antioxidant activity was measured using the DPPH method on PLBs extracts, plant extracts, and ascorbic acid as a positive control, with the determination of the inhibitory concentration 50 (IC_{50}) values. **Results:** PLB growth was observed optimally on day 60. Phytochemical examination identified the presence of compounds from the alkaloid, phenolic, tannin, flavonoid, saponin, steroid, and terpenoid groups in all samples. Antioxidant test showed that *Dendrobium crumenatum* PLBs extract had the most vigorous activity among all samples, with an IC_{50} value of 76.06 ± 0.41 µg/mL (strong category). This value was superior to that of the plant extract of *D. crumenatum*, at 175.26 ± 0.46 µg/mL. In general, PLB extracts from both species produced significantly more vigorous antioxidant activity than plant extracts. **Conclusion:** PLB culture is an effective alternative for producing Dendrobium biomass with a higher content of bioactive compounds. *D. crumenatum* PLB extract has the potential to be developed as a natural antioxidant agent.

Keywords: Dendrobium; protocorm-like bodies (PLBs); DPPH; antioxidants; secondary metabolites; IC_{50}.

Cite This Article

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INTRODUCTION

Free radicals play a crucial role in the pathogenesis of various chronic degenerative diseases; therefore, the search for safe and effective natural antioxidants from natural sources remains a key focus of global research in the health and pharmaceutical fields. Orchids of the genus Dendrobium have long been used in traditional Asian medicine due to their diverse secondary metabolite content, including phenolics, flavonoids, and alkaloids (1,2). The presence of these bioactive compounds indicates strong potential as therapeutic agents, including antioxidants.

Despite their high potential, the availability of Dendrobium species in nature is often limited, and they are threatened with extinction. Therefore, the development of alternative methods for producing biomass and active compounds is crucial. Tissue culture techniques, particularly the induction of Protocorm-Like Bodies (PLBs), offer a sustainable and controlled solution for producing large amounts of biomass without harming natural populations (3,4). Furthermore, in vitro environmental stress conditions in PLBs cultures can sometimes trigger increased synthesis of secondary metabolites, potentially producing biomass with higher biological activity than intact plants (5,6).

Although the potential of PLBs has been recognized, in-depth comparative studies on the phytochemical profile, growth, and antioxidant activity comparing PLBs with plants, especially in *Dendrobium crumenatum* and *Dendrobium liniale* species, are still limited. This study aims to bridge this gap with three main focuses: first, observing and determining the optimal growth period of PLB; second, conducting a comprehensive qualitative screening of seven classes of secondary metabolites in PLB extracts; and third, quantitatively comparing the antioxidant activity of PLB extracts with plant extracts using the DPPH method. The results of this study are expected to provide a scientific basis for utilizing Dendrobium PLB as a raw source of natural antioxidants in the health industry.

METHODS

Study design and setting

This research employed a true experimental design with a comparative approach. It involved three main stages: (1) in vitro tissue culture experiments for the induction and optimization of Protocorm-Like Body (PLBs) growth, (2) qualitative phytochemical analysis in the laboratory, and (3) in vitro antioxidant activity testing.

PLBs culture induction was conducted in the Tissue Culture Laboratory of CV. Agro Jaya Teknologi, while secondary metabolite and antioxidant bioactivity testing were conducted in the Pharmaceutical Biology Laboratory, Faculty of Medicine and Health Sciences, University of Jambi.

Population, samples and sampling

The populations in this study were the orchid species *Dendrobium crumenatum* and *Dendrobium liniale*. The samples used included initial explants in the form of mature seeds from *D. crumenatum* and *D. liniale* as the starting material for PLBs induction. PLBs extracts were prepared from induced PLBs and harvested at the optimal growth phase (60 days after planting). The plant extracts used were from the leaves of *D. crumenatum* and *D. liniale*, which served as a comparison.

Instruments and criteria

Murashige & Skoog (MS) media was used as the base medium, enriched with sucrose, agar, ascorbic acid, coconut water, and potato extract. A UV-Vis spectrophotometer was used for absorbance measurement in the DPPH test. Standard chemical reagents used for phytochemical screening (Dragendorff Reagent for alkaloids, Ferric Chloride/$FeCl_3$ for phenolics/tannins). The positive control used was ascorbic acid. The success criteria for the antioxidant test were based on the IC_{50} value (the concentration capable of inhibiting 50% of DPPH free radicals).

Procedure and data collection

1. *Protocorm-like bodies (PLBs) induction*
   Seeds are sterilized using the sodium hypochlorite method. Seeds are planted on enriched MS medium and incubated in a culture room at a temperature. PLBs growth is observed visually and morphologically on Days 0, 30, 45, and 60 DAP.
2. *Preparation of crude methanol extract*
   PLBs biomass (60 days after planting) and plant parts were dried and extracted using the maceration method with solvent methanol for 3 x 24 hours. The concentrated filtrate from the evaporation was then used for screening and antioxidant testing.
3. *Secondary metabolite screening*
   Qualitative screening was performed on PLB extracts and whole plants to detect the presence of alkaloids, phenolics, tannins, flavonoids, saponins, steroids, and terpenoids using standard phytochemical reagents and methods (7).
4. *Antioxidant activity test (DPPH)*
   A DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared. The extract samples were diluted to a series of specified concentrations. Measurement: The extract solution was mixed with the DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at a wavelength of 517 nm using a microplate reader. The percentage of free radical inhibition was calculated. The IC_{50} value was determined using a linear regression equation that relates the rate of inhibition to the logarithm of the extract concentration.

Statistical analysis

All data obtained from the results of the antioxidant activity test are presented in the form of mean ± standard deviation (SD) and processed using Microsoft Excel statistical software. Determination of the IC50 value (the concentration that inhibits 50% DPPH free radicals) was carried out through linear regression analysis on the relationship curve between the percentage of inhibition and the logarithm of the extract concentration. To test for significant differences in antioxidant activity (IC_{50} value) between the PLBs extract group, whole plant extract, and positive control, a test was performed using One-Way ANOVA. The difference is considered statistically significant if the probability value $p < 0.05$. Qualitative analysis of secondary metabolite screening is presented descriptively.

RESULTS

Protocorm-like Bodies (PLBs) growth

Observation of the growth of Dendrobium PLBs from 0 to 60 Days After Planting (DAP) showed an increase in biomass and significant morphological changes (Figure 1). At the beginning of culture (0 DAP), the explants were small, yellowish PLBs. Significant growth began to be observed at 30 DAP with the proliferation of spreading PLB tissue. PLB reached the most optimal and dense biomass at 60 DAP, marked by the formation of yellowish-green PLBs clumps, indicating the highest multiplication rate.

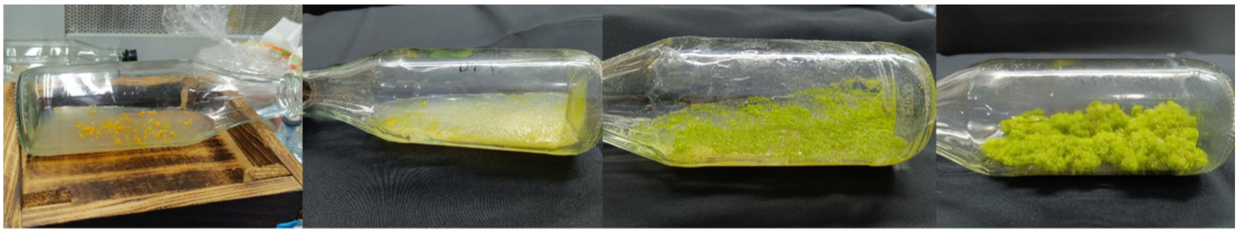

Figure 1. Proliferation and development of Protocorm-like Bodies/PLBs of *Dendrobium crumenatum* in culture media at 0, 30, 45, and 60 DAP (Days After Planting).

Secondary metabolite screening

Qualitative secondary metabolite screening showed that the PLB extract was highly rich in bioactive compounds. All seven compound groups tested were detected positively in plant and PLB extract of *Dendrobium crumenatun* and *Dendrobium liniale* (Table 1). Identified compounds included alkaloids (red precipitate), phenolics and tannins (green-blue/blue-black), flavonoids (purple), saponins, steroids, and terpenoids. The presence of phenolics and flavonoids, in particular, indicated high antioxidant potential in the cultured PLB biomass and plant of Dendrobium.

Antioxidant activity (IC_{50})

The antioxidant activity test, using the DPPH method, showed significant differences in strength among all samples (Table 2). The positive control, ascorbic acid, showed very strong activity with an IC_{50} value of 19.18 ± 0.69 µg/mL. Among the Dendrobium extracts, the PLBs extract of *Dendrobium crumenatum* showed the most vigorous activity with an IC_{50} value of 76.06 ± 0.41 µg/mL, which is classified as strong (IC_{50} < 100 µg/mL). This value is much lower than plant extract of *D. crumenatum* (175.26 ± 0.46 µg/mL, weak category) and the PLBs extract of *D. liniale* (101.22 ± 0.69 µg/mL, moderate category). Overall, PLBs culture was found to be effective in enhancing the antioxidant potential in both species.

Table 2. Inhibition values and IC_{50} of PLBs and Dendrobium plants with DPPH assay

Sample	Concentration (µg/mL)	Antioxidant Activity (% Inhibition)	IC_{50} (µg/mL)
Dendro crumenatum			
Extract PLB	250	84.71	76.06±0.41
	125	76.34	
	62.5	55.47	
	31.25	35.94	
	15.625	23.24	
Extract plant	250	63.64	175.26±0.46
	125	46.14	
	62.5	26.51	
	31.25	12.28	
	15.625	6.70	
Dendrobium liniale			
Extract PLB	250	78.51	101.22±0.69
	125	65.24	
	62.5	44.90	
	31.25	34.88	

Sample	Concentration (µg/mL)	Antioxidant Activity (% Inhibition)	IC_{50} (µg/mL)
	15.625	21.50	
Ekstrak plant	250	48.64	252.41±0.68
	125	27.86	
	62.5	16.70	
	31.25	9.68	
	15.625	4.39	
Asam askorbat	50	94.50	19.18±0.69
	25	78.77	
	12.5	42.40	
	6.25	25.69	
	3.125	10.35	

DISCUSSION

Optimal growth of PLBs at 60 days after planting indicates that the in vitro culture media conditions (nutrients) are effective in triggering the formation and proliferation of PLBs mass, making it a sustainable alternative biomass source (8,9). The results of phytochemical screening further strengthen this finding, where the detection of seven classes of secondary metabolites indicates the chemical richness of PLBs extracts. Specifically, the presence of phenolic compounds and flavonoids is key, as these classes of compounds are known to be primary natural antioxidants and are effective free radical scavengers through hydrogen atom donation (10,11). Therefore, the concentration of these compounds is expected to be positively correlated with the results of the antioxidant test.

The most important finding of this study is the superior antioxidant activity of PLBs extract compared to the plant extract. The PLBs extract of *D. crumenatum* reached an IC_{50} value of 76.06 µg/mL, twofold more potent than its entire plant extract (175.26 µg/mL). This phenomenon is consistent with *D. liniale* where PLBs also showed better activity. This increase can be interpreted as indicating that the in vitro culture conditions of PLBs, which are often considered as a controlled stress environment, have triggered the secondary metabolite biosynthesis pathway as a cell defense mechanism (12,13). The increased production of phenolics and flavonoids in PLB, as indicated by the screening results, is the main reason behind this higher antioxidant activity. In addition, the difference in strength between *D. crumenatum* and *D. liniale* can be attributed to the genetic differences between the two species in their response to the culture media.

Based on the classification, PLBs extract of *D. crumenatum* is categorized as having strong activity, showing significant potential. Although ascorbic acid (positive control) remains significantly more potent, the IC_{50} value of this extract is sufficient to warrant further development as a nutraceutical or cosmeceutical raw material. The results of this study confirm that PLBs tissue culture technique is a promising strategy for producing bioactive compounds with high antioxidant activity in a controlled manner, contributing to the fields of pharmaceutical and health biotechnology. Further research, such as isolation and identification of the structure of specific active compounds, is recommended to validate these findings clinically.

CONCLUSIONS

Based on the research results, it can be concluded that the culture of Protocorm-like Bodies (PLBs) is an effective method for producing biomass of *Dendrobium crumenatum* and *Dendrobium liniale*, with optimal growth achieved at 60 Days After Planting (DAP). Phytochemical analysis revealed a wealth of bioactive compounds, as all seven groups of secondary metabolites (alkaloids, phenolics, tannins, flavonoids, saponins, steroids, and terpenoids) were detected in the PLBs extract. The most significant finding is the superior antioxidant activity in PLBs. *D. crumenatum* PLBs extract exhibited the most vigorous activity, with an IC_{50} value of 76.06 μg/mL (strong category), which is significantly superior to that of the plant extract. This increase in activity indicates that PLBs have excellent potential to be developed as a source of natural antioxidants. For further research, it is recommended to isolate and characterize the chemical structure of the active compounds, particularly the phenolic and flavonoid groups, which are responsible for this antioxidant activity. Additionally, it is advisable to continue with bioactivity and toxicity tests for pharmaceutical validation.

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including data collection, analysis, visualization, or manuscript preparation. The authors conducted all work presented in this study manually without the assistance of AI-based tools or systems.

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