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Comparative cytotoxicity and selectivity of various orchid extracts against B16F10 melanoma cells

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Abstract

Background: Melanoma B16F10 is an aggressive form of skin cancer that requires novel therapies. This study aimed to examine the cytotoxicity and selectivity activities of five crude methanol extracts derived from three different orchid species—*Cymbidium* sp. (leaves), *Grammatophyllum speciosum* (leaves), and *Dendrobium crumenatum* (leaves, pseudobulbs, and roots) against melanoma cancer cells B16F10. **Methods:** Cytotoxicity activities were measured using MTT assay against B16F10 (cancer) and 3T3 (normal fibroblasts) cells to determine IC_{50} and selectivity index (SI). **Results:** All extracts exhibited dose-dependent cytotoxicity. The extract of *Dendrobium crumenatum* pseudobulbs showed the most vigorous activity with an IC_{50} value against B16F10 of 168.47±2.02 µg/mL. This extract also showed the best SI of 3.79 (IC_{50} 3T3 = 638.73±2.98 µg/mL), which indicates a promising selectivity potential compared to other plant part extracts. **Conclusion:** Crude methanol extract of *Dendrobium crumenatum* pseudobulbs is the best candidate as a selective anticancer agent for melanoma and requires further study to isolate its active compounds.

Keywords: Orchid; B16F10; Cytotoxicity; Selectivity; *Dendrobium crumenatum*

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INTRODUCTION

Malignant melanoma is the most aggressive form of skin cancer, originating from melanocytes, with a rising incidence globally and a high mortality rate when detected at an advanced stage (1). Despite significant advances in immunotherapy and targeted therapies, drug resistance, systemic toxicity, and high cost remain substantial challenges in melanoma treatment. Therefore, searching for new, effective chemotherapy agents, particularly those from natural sources with improved toxicity profiles, remains a top priority in oncology research.

Plants are a significant source of bioactive compounds (2). One plant family rich in secondary metabolites is the Orchidaceae (orchids), which has long been utilised in traditional Asian medicine (3). Modern phytochemical studies indicate that orchids produce a variety of compounds, including flavonoids, terpenoids, and alkaloids, which exhibit a broad spectrum of biological activities, including antioxidant, anti-inflammatory, and cytotoxic effects (4,5). However, the anticancer potential of various body parts of orchid species endemic to Indonesia remains untested.

This study was designed to investigate in depth the cytotoxic activity of five crude methanol extracts derived from three selected orchid species: *Cymbidium* sp. (leaves), *Grammatophyllum speciosum* (leaves), and *Dendrobium crumenatum* (leaves, pseudobulbs, and roots). The selection of 5 samples from different plant parts aimed to compare the distribution of active compounds within the plants. The mouse-derived cancer cell model B16F10 (melanoma) was used due to its similarity to human melanoma and is frequently used for drug screening. In addition, fibroblast cells 3T3 were used as a normal cell model to evaluate the Selectivity Index (SI). This SI measurement is crucial to assess the potential of an agent to target cancer cells without significantly damaging healthy cells.

Based on the above background, this study's main objective was to evaluate the cytotoxicity activity and SI of five crude methanol extracts of orchids against melanoma cells B16F10, by comparing their toxicity to normal cells 3T3. The results of this study are expected to identify the most promising orchid plant parts as a source of new therapeutic agent candidates for melanoma treatment.

METHODS

Study design and setting

This study used a comparative in vitro experimental design to assess and compare the cytotoxicity effects and Selectivity Index (SI) of five crude methanol extracts of orchids. The experiment was designed as a dose-response study for each extract against mouse melanoma cell lines (B16F10) and normal cells (3T3) to identify samples with the highest selective anticancer potential. This study was conducted at the Pharmaceutical Biology Laboratory, Faculty of Medicine and Health Sciences, University of Jambi for extract preparation, and the Parasitology Laboratory, Faculty of Medicine and Health Sciences, Universitas Gadjah Mada for cell culture and MTT assay stages.

Population, samples and sampling

The samples in this study were divided into two categories: plant material samples (orchid extracts), and cell strain samples target.

Plant material samples (Orchid extracts)

Target population: orchid species from the genera Cymbidium, Dendrobium, and Grammatophyllum known to have potential bioactivity. Research samples: five crude methanol extracts, including leaves (*Cymbidium* sp., *Dendrobium crumenatum*,

Grammatophyllum speciosum), pseudobulbs, and roots (*Dendrobium crumenatum*). Sampling: plant samples were collected using the purposive sampling method based on the inclusion criteria: (1) local availability in Sumatra, West Lampung, and Central Java, and (2) use of varying plant parts (pseudobulbs, leaves, roots) to assess intraspecies phytochemical potential.

Cell strain samples

Target population: Cancer cells (melanoma) and mammalian fibroblast cells. Research samples: Cancer Cells, B16F10 (Mouse melanoma, a standard model for metastatic melanoma). Normal Cells: 3T3 (Mouse fibroblast, a standard model for normal cell toxicity tests). Sampling: Cell strains were selected using convenience and purposive sampling due to their availability as standard strains (ATCC) tested in comparative toxicity studies.

Instruments and criteria

The instruments used include a rotary evaporator for evaporating methanol solvent, a laminar air flow (LAF) cabinet to maintain sterile conditions during cell culture, a CO_2 Incubator to maintain cell growth conditions (37°C, 5% CO_2), and a microplate reader to measure the absorbance of the MTT assay at 595 nm.

Cytotoxicity and selectivity criteria

The extract's effectiveness was assessed based on the following quantitative criteria of cytotoxicity (IC_{50}) and SI. The IC_{50} value indicates the concentration required to inhibit cell growth by 50%. The lower the IC_{50} value, the higher the cytotoxic potential. The potential is classified as: very strong (IC_{50} < 10 µg/mL), strong (IC_{50} = 10-100 µg/mL), moderate (IC_{50} = 100-500 µg/mL), weak (IC_{50} > 500 µg/mL). The extract is considered selective if SI > 1. SI values higher than 3 indicate promising therapeutic potential because they show much higher toxicity to cancer cells than normal 3T3 cells (6).

Procedure and data collection

1. Plant samples and chemicals

Plant samples included leaves of *Cymbidium* sp. *and Grammatophyllum speciosum*, as well as leaves, pseudobulbs, and roots of *Dendrobium crumenatum*. A total of five samples of plant material were collected from Sumatra, West Lampung, and Central Java and identified by Jatinangor Herbarium, Plant Taxonomy laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University. Sampling was carried out by purposive sampling, considering the varying phytochemical potential between plant parts. Key reagents for the cytotoxicity test were 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich), culture media (DMEM), fetal bovine serum (FBS), and antibiotics. Doxorubicin was used as a positive control.

2. Extraction procedure

Each plant part (leaves, pseudobulbs, and roots) was dried and ground into powder. A total of 200 grams of powder from each sample was extracted using the maceration method with distilled technical methanol at room temperature for 1 day. The maceration process was repeated three times. The filtrate was evaporated using a rotary evaporator at 50 °C to obtain a crude methanol extract. The yield was calculated for each extract.

3. Cell culture B16F10 and 3T3

Two cell lines were used: B16F10 (mouse melanoma cancer cells) and 3T3 (mouse normal fibroblast cells). Cells were cultured in DMEM medium supplemented with 10% FBS and 1% Penicillin-Streptomycin. Cells were maintained in an incubator at 37°C with a 5%CO_2 atmosphere.

4. Preparation of test solution

The crude methanol extract was dissolved in DMSO and diluted in culture medium to the working concentration. The final DMSO concentration in the test was kept at a level not exceeding 0.1% to avoid toxicity to the cells.

5. MTT assay test

Cytotoxicity was measured using the MTT assay in 96-well plates. B16F10 and 3T3 cells were seeded at a density of $5x10^3$ cells/well and incubated for 24 hours. Cells were then treated with five orchid extracts and a positive control (Doxorubicin) at a concentration range of 15.625—1000 µg/mL and incubated for 24 hours. After incubation, the MTT solution was added and incubated. Formazan crystals were dissolved using sodium dodecyl sulphate (SDS), and absorbance was measured using a microplate reader at a wavelength of 595 nm.

6. IC_{50} and selectivity index (SI) calculation

The percentage of cell viability was calculated based on the comparison of absorbance between treated cells and control cells. The IC_{50} value was calculated using non-linear regression analysis. The selectivity index (SI) was then calculated for each extract as the ratio of toxicity to normal cells and cancer cells:

SI=IC50 in normal cells (3T3)IC50 in cancer cells (B16F10) (1)

Statistical analysis

All tests were performed in triplicate. Data are presented as Mean ± Standard Deviation (SD) and analyzed using ANOVA with a significance level of $p<0.05$.

RESULTS

Comparative extraction results

The maceration extraction process with methanol produced five crude extracts with varying yield percentages. These differences in yield reflect the varying content of methanol-soluble metabolites in each plant part tested. The extracted yield data are presented in Table 1.

Table 1. Yield of crude methanol extract from various parts of the orchid plant

Orchid species	Plant part	Dry weight (g)	Crude extract weight (g)	Yield (%, w/w)
Cymbidium sp.	Leaves	200	24.56	12.28
Dendrobium crumenatum	Leaves	200	23.98	11.99
	Pseudobulbs	200	22.76	11.38
	Roots	200	21.65	10.83
Grammatophyllum speciosum	Leaves	200	24.69	12.34

Cytotoxicity activity and selectivity index

Inhibitory effect on B16F10 and 3T3 cells

Cytotoxicity test using MTT assay showed that the five extracts had an inhibitory effect on the proliferation of B16F10 and 3T3 cells. The IC_{50} values against cancer cells B16F10, toxicity against normal cells 3T3, and Selectivity Index (SI) are summarised in Table 2.

Table 2. IC_{50} values (µg/mL) and selectivity index (SI) of crude methanol extract of orchids against B16F10 and 3T3 cells (Mean±SD).

Sample	IC_{50} (µg/mL)±SD*		Selectivity Index/SI (3T3/B16F10)
	B16F10	3T3	
Cymbidium sp.			
Leaves	763.80±2.32	973.76±1.80	1.27
Dendrobium crumenatum			
Leaves	386.37±1.89	466.45±3.06	1.21
Pseudobulbs	168.47±2.02	638.73±2.98	3.79
Roots	277.58±2.24	384.33±1.86	1.38
Grammatophyllum speciosum			
Leaves	616.75±1.31	838.67±2.04	1.36
Doxorubicin	0.32±1.06	2.32±2.45	7.25

Based on Table 2, the extract of *Dendrobium crumenatum* pseudobulbs is the most potent extract against B16F10 cells with the lowest IC_{50} value, 168.47±2.02 µg/mL. This value places the extract in the category of moderate cytotoxicity activity (100-500 µg/mL). The extracts of *D. crumenatum* leaves and *D. crumenatum* roots also showed higher activity compared to the other two species extracts (IC_{50} = 386.37±1.89 µg/mL and 277.58±2.24 µg/mL, respectively).

In contrast, the extract of *Cymbidium* sp. leaves. showed the weakest activity with IC_{50} = 763.80±2.32 µg/mL, classified as weak activity (IC_{50} > 500 µg/mL). Regarding selectivity, the *D. crumenatum* pseudobulbs extract also showed the highest SI value of 3.79. This value, which is well above 1, indicates the ability of the extract to selectively kill B16F10 cells with lower toxicity to normal 3T3 cells (IC_{50} = 638.73±2.98 µg/mL). The positive control, Doxorubicin, showed very strong activity (IC_{50} = 0.32±1.06 µg/mL) and the highest SI (7.25).

DISCUSSION

The yield percentage of crude methanol extract was in a narrow range of 10.83% - 12.34%. *Grammatophyllum speciosum* leaf extract gave the highest yield of 12.34%, while *Dendrobium crumenatum* root extract gave the lowest yield of 10.83%. However, no positive correlation was found between high yield and cytotoxicity potential against B16F10 cells. For example, *Grammatophyllum speciosum* leaf extract, which had the highest yield, actually showed weak activity against B16F10 (IC_{50} = 616.75±1.31 µg/mL). In contrast, the extract of *Dendrobium crumenatum* pseudobulbs, which had an intermediate yield (11.38%), showed the strongest activity (IC_{50} = 168.47±2.02 µg/mL) and the best selectivity (SI = 3.79). This indicates that the anticancer activity is determined by the quality and specific structure of the secondary metabolites, not only by the total quantity of the extracted material. The relatively stable yields between

plant parts indicate consistent extraction efficiency, but the biological activity is highly dependent on the plant part used.

The cytotoxic activity of crude methanol extract of orchids against mouse melanoma cells showed significant variation between plant parts. Based on the classification criteria of crude extract cytotoxic activity (7), *Dendrobium crumenatum* pseudobulbs extract (IC_{50} = 168.47±2.02 µg/mL) and *D. crumenatum* root extract (IC_{50} = 277.58±2.24 µg/mL) were classified as having moderate activity (IC_{50} between 100-500 µg/mL). This moderate activity provides a strong initial indication that the pseudobulbs and roots of *D. crumenatum* contain higher or more potent concentrations of bioactive compounds than the other extracts tested. On the other hand, leaf extracts from all three species showed weaker activity (IC_{50} 386.37±1.89 µg/mL), with the *Cymbidium* sp. leaf extract being the lowest (IC_{50} = 763.80±2.32 µg/mL). This difference in cytotoxicity potential is also reflected in the value of Doxorubicin as a positive control, which showed potent activity (IC_{50} = 0.32±1.06 µg/mL) because it is a pure compound.

These results emphasise the importance of comparative evaluation between different plant parts of the same species. In *Dendrobium crumenatum*, the order of cytotoxicity potency was pseudobulbs (IC_{50} 168.47±2.02 µg/mL), roots (IC_{50} 277.58±2.24 µg/mL), leaves (IC_{50} 386.37±1.89 µg/mL). These differences may be related to the phytochemical profile and distribution of secondary metabolites. Pseudobulbs and roots, as storage organs, often accumulate specific secondary metabolites (such as frequently found in Dendrobium) as a defence mechanism or energy storage (8,9). This accumulation results in higher concentrations of cytotoxic compounds in pseudobulb extracts compared to leaves, which may be more dominant in photosynthetic compounds (such as chlorophyll) that are less cytotoxic.

The most crucial aspect of this study was the assessment of the selectivity index (SI), which compares the toxicity to cancer cells (B16F10) and normal cells (3T3). The *D. crumenatum* pseudobulb extract showed the highest SI of 3.79. A SI value > 3 is considered a promising indication of selectivity potential (10), as it indicates that the extract is 3.79 times more toxic to melanoma cells than to normal fibroblast cells 3T3. This selectivity implies that the active compounds in pseudobulbs are likely not only generally toxic, but also act through mechanisms that target specific proliferation or survival pathways that are dominant in melanoma B16F10 cells. These targets may include the MAPK pathway, apoptosis induction, or tyrosinase inhibition, often found in melanoma. Compounds from the alkaloids, phenanthrenes, or terpenoids groups can inhibit the growth of melanoma cells through the MAPK pathway, apoptosis induction, or tyrosinase inhibition (11–13). Doxorubicin's toxicity to 3T3 cells was also relatively low (IC_{50} = 2.32±2.45 µg/mL), resulting in the highest SI (7.25), highlighting that although the crude orchid extract is not as effective as the pure drug, the SI=3.79 value is still auspicious for a natural product.

This study identifies *D. crumenatum* pseudobulbs as the most promising source for isolating selective anticancer agents against melanoma B16F10. This potential is directly attributed to the pseudobulbs' unique secondary metabolite content. For further research, an essential step is performing bioassay-guided fractionation on pseudobulb extracts to isolate and identify specific active compounds. In addition, mechanism-of-action assays (e.g., apoptosis and cell cycle analysis) are needed to validate how these compounds can selectively kill B16F10 cells.

CONCLUSIONS

This study successfully evaluated and compared the cytotoxicity and selectivity activities of five crude methanol extracts of orchids against melanoma cancer cells B16F10 and normal cells 3T3 using the MTT assay. The results showed that the pseudobulb extract of *Dendrobium crumenatum* was the most potent against B16F10 cells with the lowest IC_{50} value of 168.47±2.02 µg/mL, placing it in the moderate activity category. Most importantly, this extract also showed the highest Selectivity Index (SI) 3.79 (SI > 1), indicating that this extract is selectively more toxic to B16F10 cells than normal cells 3T3. In conclusion, the crude methanol extract of *D. crumenatum* pseudobulbs is the most promising candidate among the samples tested. It provides a strong scientific basis for fractionation studies and isolation of active compounds in the search for new anticancer agents for the treatment of melanoma.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of 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 utilised at any stage of this study, including data collection, analysis, visualisation, 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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