

Cytotoxicity of three Orchid species extracts against HeLa, MCF-7, and T47D cancer cells

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Abstract

Background: Cancer remains a leading cause of death globally, necessitating the search for novel therapeutic agents from natural sources. Orchids, known to produce diverse secondary metabolites, hold significant potential. **Objective:** This study aims to evaluate and quantitatively compare the in vitro cytotoxicity activity of crude methanol extracts from three orchid species against HeLa, MCF-7, and T47D cells using the MTT assay. **Methods:** This study investigated the comparative in vitro cytotoxic activity of crude methanol extracts from three native orchid species, *Cymbidium* sp., *Grammatophyllum speciosum*, and *Dendrobium crumenatum*, against three human cancer cell lines: HeLa (cervical), MCF-7 (breast), and T47D (breast). Cytotoxicity assessment was performed using the MTT Assay. The extracts were tested at various concentrations to determine their inhibitory concentrations IC₅₀. **Results:** The results showed that the three orchid extracts exhibited varying cytotoxic effects across the tested cell lines. Significantly, the crude methanol extract of *Dendrobium crumenatum* showed the most promising activity, especially against MCF-7 breast cancer cells, with an IC₅₀ value of 126.58±5.50 µg/mL. The activity against HeLa and T47D was less potent. These findings indicate that secondary metabolites in *Dendrobium crumenatum* have selective antiproliferative properties. **Conclusion:** In conclusion, the methanol extract of *Dendrobium crumenatum* is a potential candidate for the isolation and further identification of active compounds in developing anticancer drugs, especially for breast cancer.

Keywords: Orchid, Cytotoxicity; MTT Assay; MCF-7, *Dendrobium crumenatum*

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INTRODUCTION

Cancer remains a serious global health problem and a leading cause of death worldwide. Despite significant advances in conventional treatments (such as chemotherapy, radiotherapy, and immunotherapy), drug resistance, severe side effects, and high treatment costs remain substantial challenges (1). Therefore, the search for new therapeutic compounds that are more effective, selective, and have minimal toxicity to normal cells remains a primary focus in biomedical research. Natural resources, predominantly plants, have proven to be a rich repository of bioactive compounds, inspiring over 60% of currently available anticancer drugs (2).

In the context of the search for new compounds, the Orchidaceae (orchid) family has attracted considerable attention. Orchids are the second largest and most widespread family of flowering plants and have long been used in traditional medicine across various cultures, not only as ornamental plants (3). Modern phytochemical research indicates that orchids are rich in a wide variety of secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenols, which are known to possess important biological activities, including anti-inflammatory, antioxidant, antibacterial, and anticancer activities (4). However, the cytotoxic potential of many orchid species in Indonesia remains inadequately explored.

This study specifically focuses on the cytotoxic potential of crude methanolic leaf extracts of three orchid species: *Cymbidium* sp., *Grammatophyllum speciosum*, and *Dendrobium crumenatum*. These three species were selected based on local ethnobotanical data or their significant secondary metabolite richness (5). To evaluate the spectrum of activity, the extracts were tested against three different human cancer cell lines, namely HeLa (cervical cancer), MCF-7 (breast cancer), and T47D (breast cancer) cells. This cytotoxicity test is essential to compare the activity between orchid species and the selectivity of the extracts against various types of cancer cells.

Based on the above background, this study aims to evaluate and quantitatively compare the in vitro cytotoxicity activity of crude methanol extracts from three orchid species (*Cymbidium* sp., *Grammatophyllum speciosum*, and *Dendrobium crumenatum*) against HeLa, MCF-7, and T47D cells using the MTT Assay. The results of this study are expected to provide a scientific basis and justification for the further development of the most active orchid species as a source of new chemotherapeutic agents.

METHODS

Study design and setting

This study adopted an in vitro experimental design, in which the cytotoxic activity of three plant extracts (crude methanol extracts of *Cymbidium* sp., *Grammatophyllum speciosum*, and *Dendrobium crumenatum* leaves) was quantitatively tested against the proliferation of three human cancer cell lines. This study design focused on a dose-response comparison to determine the IC₅₀ value of each extract on each cell line. This study was conducted at the Parasitology Laboratory, FKK-MK UGM for the cell culture and MTT assay stages. Meanwhile, the extraction and sample preparation processes were carried out at the Pharmaceutical Biology Laboratory, FKIK, Jambi University. All procedures followed standard protocols for cell culture and toxicity testing.

Population, samples and sampling

The samples in this study consisted of two main components: plant material (orchid leaves) and human cancer cell lines.

Plant material samples (orchid populations and samples)

The target populations of plant material were orchid species with phytochemical and ethnobotanical potential for medicinal purposes. Three species were selected as research samples: *Cymbidium* sp., *Grammatophyllum speciosum*, and *Dendrobium crumenatum*. Sampling was conducted using purposive sampling. Leaf samples were collected and botanically identified to ensure taxonomic validity.

Cell line samples (cancer cell populations and samples)

The populations in this context are human cancer cells relevant to prevalent diseases. Three human cancer cell lines were selected as target samples: HeLa (cervical cancer cells), MCF-7 (breast cancer cells, estrogen receptor positive), and T47D (breast cancer cells, estrogen receptor positive). These cell lines were selected (Sampling) using a purposive sampling method due to their representation as standard strains frequently used in cytotoxicity assays and their clinical relevance as models for breast cancer (MCF-7 and T47D) and cervical cancer (HeLa). These cells were then cultured under controlled conditions.

Instruments and criteria

Any instrument or questionnaire used in the study must be explained in detail, including general information, total questions, scale or category, reporting criteria, reference, and how its validity and reliability were ensured.

Procedure and data collection

1. Plant materials and chemicals

Leaves from three orchid species, *Cymbidium* sp., were collected from Sumatra, *Grammatophyllum speciosum* from West Lampung, and *Dendrobium crumenatum* from Temanggung, Central Java. Specimen identification was performed by Jatinangor Herbarium, Plant Taxonomy laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The solvent used for extraction was technical methanol. The primary reagents in the cytotoxicity test are 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich), culture medium DMEM, RPMI, Fetal Bovine Serum (FBS) (Gibco), and the antibiotic Penicillin-Streptomycin (Gibco). The positive control drug used is Doxorubicin.

2. Preparation of cancer cell cultures

Three human cancer cell lines, HeLa (cervical cancer), MCF-7 (breast cancer), and T47D (breast cancer), were obtained from the Parasitology Laboratory, Faculty of Medicine, Public Health, and Nursing (FK-KMK) UGM. The cells were cultured in DMEM and RPMI medium enriched with 10% FBS and 1% Penicillin-Streptomycin at 37 °C incubation conditions with a 5% CO₂ atmosphere and high relative humidity. The cells were maintained until they reached 80% confluence before being used for testing.

3. Crude methanol extraction procedure

Fresh orchid leaves were cleaned, dried in the dehydrator, and ground into powder. Five hundred grams of leaf powder were macerated with methanol technical (1:10) for 24 hours at room temperature. The maceration process was repeated three times. The collected filtrate was evaporated using a rotary evaporator at 50 °C to remove methanol, producing a crude methanol extract (crude methanol extract). The yield of the extract was calculated, and the extract was then stored in a refrigerator until use.

4. Cytotoxicity test with MTT assay

The cytotoxicity test was performed using the MTT method. Cells were seeded on 96-well plates at a density of 5x10³ cells/well and incubated for 24 hours to allow cell

attachment. Then, the cells were treated with orchid extract at various concentrations, ranging from 15.625–1000 µg/mL. Cell controls (cells and media only), media controls, and positive controls (doxorubicin) were also included. Cells were incubated for 24 hours. After incubation, the medium was discarded, and MTT solution (5 mg/mL) was added to each well. The plate was incubated for 4 hours. After that, SDS is added to dissolve the formed formazan crystals. The absorbance is measured using a microplate reader at a wavelength of 595 nm.

Statistical analysis

Cytotoxicity calculation

The percentage of cell viability was calculated using the following formula:

$$\text{Viability \%} = \frac{\text{Sample Absorbance} - \text{Blank Absorbance}}{\text{Control Absorbance} - \text{Blank Absorbance}} \times 100\% \quad (1)$$

The inhibitory concentration value 50% (IC₅₀), which is the concentration that inhibits cell growth by 50%, was determined from the linear regression curve between the extract concentration and the percentage of cell viability. All tests were conducted in triplicate. Data were analyzed statistically using ANOVA with a significance level of $p < 0.05$. The statistical software used is SPSS.

Selectivity index (SI)

The selectivity index (SI) value is calculated using the formula:

$$\text{SI} = \frac{\text{IC}_{50} \text{ in normal cells (Vero)}}{\text{IC}_{50} \text{ in cancer cells (HeLa/MCF-7/T47D)}} \quad (2)$$

A cytotoxicity test was performed on normal Vero cells (African green monkey kidney epithelial cells) to assess the extract's safety. The IC₅₀ results against Vero cells and the Selectivity Index (SI) calculations against the three cancer cell lines are presented in Table 2. SI values > 2 or > 3 generally indicate good selectivity potential.

RESULTS

Cytotoxicity evaluation of extracts against human cancer cells

Based on Table 1, the methanol extracts of the three species showed varying cytotoxicity activities. *Dendrobium crumenatum* extract exhibited the most vigorous activity among the three extracts, especially against MCF-7 cells with an IC₅₀ value of 126.58±5.50 µg/mL. In general, the activity of the *D. crumenatum* extract was higher than that of *Grammatophyllum speciosum*. In addition, it was found that MCF-7 cells tended to be more sensitive to orchid extract than T47D cells.

Table 1. IC₅₀ values of orchid leaf extracts against MCF-7 cancer cells.

Sample	IC ₅₀ (µg/mL)±SD*		
	HeLa	MCF-7	T47D
<i>Cymbidium</i> sp.	336,89±3.35	187,59±6.09	487,61±5.17
<i>Dendrobium crumenatum</i>	318,97±3.36	126,58±5.50	551,94±4.13
<i>Grammatophyllum speciosum</i>	287,87±2.33	147,49±4.00	647,67±4.61
Doxorubicin	0,24±3.11	0,46±2.14	0,78±2.38

Remarks: IC₅₀ values are reported as the mean ± SD of three independent assays, * IC₅₀ values (µg/mL) yield four cytotoxic categories: high ≤ 20 , moderate 21-200, weak 201-500, and non-toxic > 500 .

Toxicity to normal cells and selectivity index (SI)

Based on Table 2, the IC₅₀ of all extracts against normal Vero cells ranged from 395.13 to 524.71 (µg/mL), indicating lower toxicity than cancer cells. The SI value showed that *Dendrobium crumenatum* extract had the highest selectivity, especially against MCF-7 cells with a SI of 3.79. MCF-7 cells were the most selective target among the three cancer cell lines tested.

Table 2. Selectivity Index (SI) values of orchid leaf extracts against vero cells

Sample	IC ₅₀ (µg/mL) Vero	SI vs. HeLa (Vero/HeLa)	SI vs. MCF-7 (Vero/MCF-7)	SI vs. T47D (Vero/T47D)
<i>Cymbidium sp.</i>	524,71	1,56	2,80	1,08
<i>Dendrobium crumenatum</i>	480,09	1,51	3,79	0,87
<i>Grammatophyllum speciosum</i>	395,13	1,37	2,68	0,61
<i>Doxorubicin</i>	16,34	68,10	35,53	20,95

DISCUSSION

In vitro cytotoxicity testing results (Table 1) showed that the crude methanol extracts of the three orchid species exhibited inhibitory activity against cancer cell proliferation, with varying potency levels depending on the orchid species and the target cancer cell strain. Based on the crude extract IC₅₀ cytotoxicity criteria (e.g., Meyer, 1982 or Geran, 1972) which classifies vigorous activity at IC₅₀ < 100 µg/mL, moderate activity at IC₅₀ = 100-500 µg/mL, and weak activity at IC₅₀ > 500 µg/mL, the orchid extracts in this study mostly showed moderate cytotoxic activity against HeLa and MCF-7 cells.

Specifically, *Dendrobium crumenatum* extract showed the greatest potency with the lowest IC₅₀ (126.58 ± 5.50 µg/mL) against MCF-7 cells. This confirms the hypothesis of the anticancer potential of this species. On the other hand, T47D cells appeared more resistant to all extracts, with most IC₅₀ values approaching or exceeding 500 µg/mL. For example, the IC₅₀ of *Grammatophyllum speciosum* to T47D was 647.67±4.61 µg/mL, classified as weak activity.

Variations in cytotoxic activity between orchid species may be attributed to differences in secondary metabolite content. For example, the IC₅₀ of *Grammatophyllum speciosum* against MCF-7 (147.49 µg/mL) was lower than that of *Cymbidium sp.* (187.59 µg/mL), suggesting a possible higher concentration or type of active compound in *Grammatophyllum speciosum* (4,6).

Furthermore, differences in cancer cell sensitivity (selectivity) were also evident. MCF-7 cells were consistently the most sensitive to all three extracts. This difference is likely due to the specific molecular characteristics of each cell line. For example, MCF-7 and T47D are ER+ breast cancer cells, but T47D often exhibits different drug resistance mechanisms or differences in target protein expression compared to MCF-7 (7)The extract's selectivity against MCF-7 (supported by SI=3.79) suggests that the active compounds in *Dendrobium crumenatum* may target specific signaling pathways that are active in MCF-7 cells with wild-type p53 but less effective in T47D cells with mutated p53 (8,9).

A crucial aspect in evaluating anticancer agents is the Selectivity Index (SI), where a value of SI > 1 indicates that the extract is more toxic to cancer cells than to normal cells. The results in Table 2 show that *Dendrobium crumenatum* extract has the highest SI value of 3.79 against MCF-7 cells. An SI > 2 value is generally considered a threshold indicating promising selectivity (10). This high selectivity implies that the bioactive compounds in *D. crumenatum* may work through mechanisms specifically

targeting proliferation or signaling pathways unique to MCF-7 cells, related to estrogen receptor expression or the MAPK pathway (11,12), while remaining relatively unaffected by normal Vero cells. In contrast, *Grammatophyllum speciosum* extract showed suboptimal SI values ($SI < 1$) against T47D and HeLa cells, suggesting that its toxicity is nonspecific or even more toxic to Vero cells (as in T47D with $SI = 0.61$). Although the SI value of orchid extract is still much lower than Doxorubicin ($SI = 35.53$ in MCF-7), the finding of 3.79 in *D. crumenatum* provides strong justification to continue fractionation studies and isolation of pure compounds from this species.

CONCLUSIONS

This study successfully evaluated the in vitro cytotoxicity potential of crude methanol extracts of three orchid species (*Cymbidium* sp., *Grammatophyllum speciosum*, and *Dendrobium crumenatum*) against three human cancer cell lines (HeLa, MCF-7, and T47D) using the MTT Assay. The results showed that all extracts had varying and dose-dependent cytotoxic activity. Comparatively, crude methanol extract of *Dendrobium crumenatum* leaves was the most active, specifically against MCF-7 breast cancer cells with the lowest IC_{50} value of 126.58 ± 5.50 $\mu\text{g/mL}$, classified as moderate activity. In conclusion, *Dendrobium crumenatum* extract showed the most promising potential among the tested samples. It can be used as a viable natural source for further development in discovering new anticancer agents. In conclusion, *Dendrobium crumenatum* extract was the most active. It showed the best selectivity ($SI = 3.79$) against breast cancer cells MCF-7 among the tested samples, making it a viable natural resource for further development in discovering new anticancer agents.

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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