Bidara *(Ziziphus mauritiana Lam.)* leaves as Cytotoxic agent Against Hela Cervical Cancer Cell Line

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Abstract. Cancer remains as one of the leading cause of fatal disease globally. Though preventable by vaccines, cervical cancer remains the second most common type of cancer diagnosed in women. Current cancer treatments are costly, time-consuming, and gave various side effects. Bidara plants contain secondary metabolites and antioxidants that are beneficial for health. This study aims to examine the anticancer effects of bidara leaves methanolic extract on HeLa cervical cancer cells. We incubate HeLa cells with different doses of bidara leaf extract (0 µg/ml, 15.6 µg/ml, 31.3 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml) for 24 hours and determined the percent viability of cells using MTT assay with absorbancy at 595nm. We found that bidara leaves methanolic extract has IC_{50} of 23 µg/ml which means that bidara leaves has strong anticancer potential against Hela cancer cell line. We did in-silico analysis on two leading compound in bidara leaves extract, desmethylclomipramine and demeclocycline where we find that those two compounds have binding affinity with BCL-2, an important protein involved in apoptosis process with molecular affinity energy ranging from -6.5 kcal/mol and -7.1 kcal/mol respectively which indicates good affinity binding between both compounds and BCL2.

1 Introductions

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Cancer, one of the leading causes of death globally, is caused by cells that mutate and divide uncontrollably, invading surrounding tissues and other organs in the body. In Indonesia in 2020 alone, as many as 396,314 people were diagnosed with cancer, with cancer-related deaths recorded at 234,511 people per year.[1] Based on gender, breast cancer and cervical cancer are the two most common types of cancer found in women.[1] According to World Cancer Research Funds, Indonesia ranked the 3rd country with the highest cervical cancer incidence, with 36,964 new cases and over 20,000 deaths in 2022 alone.[2] Cancer treatment currently includes invasive methods through surgery, radiotherapy, and chemotherapy, which have resulted in a decrease in the quality of life of the patient as well as financial losses and a high mental burden for both cancer patients and their families.

Bidara plant (*Ziziphus mauritiana Lam*.) is one of the plants used in traditional medicine. The bidara plant is known to contain compounds such as glycosides, alkaloids, flavonoids, terpenoids, saponins, and pectin A. Bidara plant has been used as a traditional folk medicine to treat nausea, diarrhea, dysentery, fever, wound treatment, and anti-inflammatory [3] Bidara leaves themselves have been reported to have anticancer effects on lung cancer cells of type A549 by increasing apoptosis and preventing the proliferation of cancer cells.[4] Tabassam *et al* (2021) reported that ziziphus mauritiana exhibit anticancer effect by inhibiting cell growth and tumor initiation process against breast cancer cells (SKBR3) and ovarian cancer cells (SKOV3).[5] Research by Lestari *et al* (2025) reported that Ziziphus mauritiana leaf extract has anticancer effect on triple negative breast cancer cell line MDA-MB-231 by downregulating CD81 resulting in cytotoxic effects that reduce cell viability and increase intracellular ROS levels in a dose-dependent manner.[6] In cancer cells, ROS accumulation occurs, which causes adaptation to cancer cells and encourages migration and invasion of cancer cells and resulting in cancer cell metastases. In cancer cells, an increase in ROS in the cell results in a cellular response that causes a change in cell metabolism from normal metabolism in the mitochondria to glycolysis metabolism in the cytoplasm, which accelerates the process of ATP synthesis to facilitate cancer progression.[7]

Through previous research, we have found that bidara leaf extract obtained from the Ungasan area in Bali has high levels of antioxidants and secondary metabolites.[8] Among the compounds found in the extract, we found desmethylclomipramine and demeclocycline, which have potential as anticancer compounds. [9-11] However, there has not been much research exploring the potential of bidara leaves in inhibiting the growth of HeLa cervical cancer cells. In this study, we want to know if bidara leaves have an anticancer effect on HeLa cervical cancer cells, and using molecular docking, we want to predict what role does desmethylclomipramine and demeclocycline, have on P53, a protein that has an importantr role apoptosis pathway.

2 Research Method

2.1 HeLa Cells Preparation

HeLa cells used in this study were obtained from the cell line collection from the Faculty of Medicine and Health Sciences biomolecular laboratory at Warmadewa University in Bali, Indonesia. HeLa cells were sub-cultured using DMEM+10% FBS

in flask before seeded in 96 well plate with confluency of 10,000 cells/well. Afterward, the plate was incubated for another 24 hours at 37°C, 5% CO_2 cell culture incubator (Newbrunswick CO2 Incubator) to ensure that all the cells has attached well onto the bottom of the wells.

2.2 MTT Cytotoxic Assay

2.2.1 Bidara Extract Dose Variation

Dose variation of bidara extract was done by adding 1 mg of bidara leaf methanol extract to 1ml of DMEM medium to obtain 1,000μg/ml concentration. Serial dilution was carried out to obtain concentrations of 500μg/ml, 250μg/ml, 125μg/ml, 62.5μg/ml, 31.3μg/ml, and 15.6μg/ml and stored at 4°C.

2.2.2 MTT Assay

On the 96-well plate with cells, regular DMEM media was replaced by different dose media prepared and treated for 24 hours. After 24 hours, the culture medium is replaced with DMEM medium containing 10% MTT solution and incubated for 2-4 hours at 37°C with 5% CO_2 using (Newbrunswick CO2 Incubator) cell culture incubator. Living cells will transform MTT into a bluish-purple formazan. The principle of the MTT assay measures cell viability by measuring the amount of formazan produced from MTT in the mitochondria of living cells. After incubation, the MTT solution was removed, the cells were rinsed with PBS, and DMSO was added and gently micxed to dissolve the formazan. After incubating for 5 minutes to ensure the formazan was dissolved, we measured the absorbance of the solution at a wavelength of 595 nm using a UV-spectrophotometer (Biochrom EZ Read 2000).

2.2.3 Cell Cytotoxic Analysis and IC_{50} Determination

The analysis of the MTT cytotoxic assay and the determination of IC_{50} were carried out by calculating the average absorbance of cells at each dose of bidara and calculating the average number of cells still viable after treatment. Calculation of % viability was done using the formula % viability of cells = ((OD dose / OD of control) x 100), and the determination of IC_{50} was carried out by calculating the dose where there was only half the number of HeLa cells compared to the negative control. In this study, IC_{50} calculation was carried out using the IC_{50} Calculator from AAT Bioquest11.

2.3 In-Silico Molecular Docking

Some studies indicate that the objective is to develop innovative medicines by enhancing the understanding of phytochemical mechanisms that interact with targets to block or activate certain proteins and enzymatic pathways for the treatment of particular diseases. Molecular docking aims to enhance the prediction of ligand-receptor complex configurations through computational methods.[12] The

compounds obtained from Bidara extract, as indicated in our previous studies, include demeclocycline and desmethylclomipramine. (8)

The AutoDock 4.2.6 software was downloaded from the official Scripps Research Institute website (http://autodock.scripps.edu/) to utilize the docking method. The processed protein molecule was imported into the AutoDock 4.2.6 workspace. After adding polar hydrogen atoms, the Kollman and Gasteiger charges of the protein were calculated. The protein was then saved in PDBQT format and used as the target. The grid centers for each protein were selected to align with their active sites. The optimal protein–ligand conformations were determined based on their maximum binding affinities using AutoDock 4.2.6. Interactions between ligands and proteins were visualized using the Discovery Studio client 2021, and three-dimensional protein–ligand configurations were analyzed to investigate binding mechanisms. [12]

3 Results and Discussions

3.1 MTT Cytotoxic Assay

Absorbance readings using spectrophotometry with a wavelength of 595 nm were performed after 24 hours of incubation with bidara leaf extract and then analyzed to obtain the % viability. Based on the results obtained, it was observed that the number of living HeLa cells after incubation was inversely proportional to the concentration of the bidara extract administered, where the higher the bidara dose, we observe lower the number of viable cells in the well. As seen in Table 1, even at the lowest dose of 15.6 µg/ml, only 54% of the HeLa cells remained alive, while at the highest dose of 1000 µg/ml, only 4% of the HeLa cells survived. This shows that bidara extract has an anticancer effect that is dose-dependent against HeLa cervical cancer cell lines. The result in this study correlates with findings by Lestari *et al* (2025) where they find that *ziziphus mauritiana* extract produced cytotoxic effects against triple triple-negative breast cancer MDA MB-231cell line in a dose-dependent manner, where they also found that the higher the extract concentration results in lower cell viability.[6]

Table 1. Results of % Viability of HeLa Cells Using MTT Test.

Bidara Dose (µg/ml)	% HeLa Cell Viability
0	100%
15.6	54%
31.3	48%
62.5	37%
125	25%
250	16%
500	16%

1000	4%

3.2 Determination of IC50

Based on the % cell viability results in Table 1 above, we constructed a graph of % cell viability. From the graph, we observed a trend that cell viability decreases as bidara extract dose increased as seen in Figure 1. Using the AAT Bioquest11 IC50 calculator, we calculated IC_{50} of bidara extract against HeLa to be at 23 μg/ml. [13], According to journal by Suwarman *et al.* [14], the IC_{50} value that we obtain in this study falls under "strong" since it falls in the range of 10-100μg/ml. This finding also relates with findings by Lestari et al (2025) where they found that treatment of ziziphus mauritiana extract significantly reduces cell viability in triple negative breast cancer MDA MB-231cell line. [6]

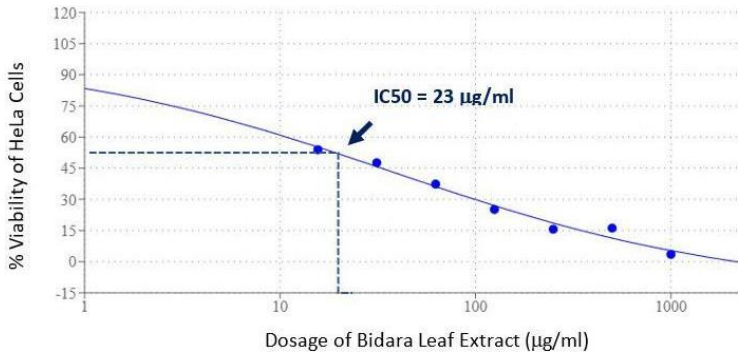

Fig 1. % Viability of HeLa Cells Post Incubation 24 Hours Bidara Leaf Extract.

3.3 In-Silico Molecular Docking

Compound that we get from Bidara Extract from our previous study before is Demeclocyline dan desmethylclomipramine (DCMI).(8) Demeclocycline been observed to inhibit certain cellular processes, potentially affecting cancer cell proliferation. Demeclocycline has been shown to inhibit specific biological processes, potentially affecting cancer cell proliferation. A study by Sarkar *et al*. in 2020 found that demeclocycline directly inhibited human BTIC (Brain Tumor-Initiating Cells) development by acting as a new inhibitor of BTIC proliferation via both direct and indirect monocyte activation.[9] Another study by Borbone *et al*. in 2015 found that desmethylclomipramine inhibits the growth of lung cancer stem cells (CSCs), diminishes their stemness capabilities, and enhances the effectiveness of conventional chemotherapy drugs (10).

These two ligands/compounds were screened from Bidara extract and docked against BCL2 as a target protein. It is well-established members of the B-cell lymphoma 2 (BCL-2) protein family have been the focus of much research in the past ten years because of their importance in regulating carcinogenesis, apoptosis,

and cellular responses to anticancer treatment. BCL 2 is one of the proteins that has the ability to either promote or inhibit apoptosis [15].

In this study we found that both compounds show good result with docking scores, desmethylclomipramine has score of -6,5 while demeclocyline has score of -7,1 binding affinity value with protein BCL2 respectively. In molecular docking, a negative score signifies a favorable interaction, with more negative values indicating stronger binding. Therefore, the lowest (most negative) binding score suggests the highest likelihood of a stable and strong interaction between the protein and the ligand. This means that despite both compound having a good affinity toward BCL2 receptor, demeclocycline has stronger affinity compared to desmethylclomipramine toward BCL2 receptor.

Table 2. Binding affinity of ligand molecules with BCL2 receptor

Ligand molecule	ΔG(kcal/mol)	Rmsd lb	Rmsd ub
desmethylclomipramine	-6.5	0.000	0.000
demeclocyline	-7.1	0.000	0.000

Note : RMSD root mean square deviation; RMSD/lb Rmsd lower bond; RMSD/ub Rmsd upper bond.

4 Research Conclusion

Anticancer effect of bidara leaf extract on HeLa cells study showed promising results, where the number of viable HeLa cells decreased with the increase in the dose of bidara leaf extract with IC_{50} of 23 μg/ml, which indicates that bidara extract had strong cytotoxic effects on HeLa cells. The findings of this study offer scientific support for the traditional application of bidara extract in cancer therapy. The bidara plant, which is easy to find and inexpensive, has the potential to be an essential ingredient in making anticancer drugs that are affordable for cancer patients and easy to obtain. Furthermore, based on in silico molecular docking, desmethylclomipramine and demeclocycline, two compounds found in bidara extract, were found to have as score of -6.5 and -7.2 which indicate a strong molecular affinity to BCL2, a protein that is involved in the intrinsic pathway of apoptosis. However, further research is still needed to isolate the active compounds in bidara extract and understand its mechanism against cervical cancer cells. In addition, since this study does not use normal cell control, further research is needed to see the effect of bidara leaves on normal cells to ensure the selectivity of cytotoxic activity of bidara leaf extract.

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