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In Vitro Activity Of Apigenin And Luteolin Of Durian Peel (Durio Zibethinus Murray) Induced Apoptosis In Cervical Cancer And Molecular Docking Approach: Involvement Of Bcl-2 Pathways

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Abstract

Cervical cancer is the fourth most common cancer among women, with resistance to therapy often linked to Bcl-2 overexpression. This study evaluated the anticancer activity of durian peel extract (Durio zibethinus Murray) against HeLa cells through Bcl-2-mediated apoptosis and investigated apigenin and luteolin interactions with Bcl-2 via molecular docking. The extract was prepared by ethanol maceration, characterized by phytochemical screening and GC-MS, and tested for cytotoxicity using the MTT assay, while apoptosis was assessed through Bcl-2 immunofluorescence. Phytochemical screening confirmed flavonoids, alkaloids, saponins, tannins, and triterpenoids, with GC-MS identifying over 93 bioactive compounds. The extract exhibited significant cytotoxicity with an IC_{50} of 5.92 µg/mL, and cell viability decreased concentration-dependently ($p < 0.05$), accompanied by apoptotic morphological changes and reduced Bcl-2 expression. Molecular docking revealed favorable binding of apigenin and luteolin to Bcl-2, with binding energies of −6.01 and −5.55 kcal/mol, respectively. In conclusion, durian peel extract demonstrates promising anticancer activity against HeLa cells by inducing apoptosis through Bcl-2 suppression, with apigenin and luteolin serving as potential natural candidates for cervical cancer therapy.

Keywords: ***Apigenin, Luteolin, Apoptosis, Cervical Cancer, Durio Zibethinus, Bcl-2***

INTRODUCTION

Cervical cancer remains a major global public health challenge and is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer-related mortality among women worldwide. According to the Global Cancer Observatory (GLOBOCAN) published by the International Agency for Research on Cancer (IARC), the global burden of cervical cancer continues to increase. In 2020, an estimated 604,127 new cases and 342,186 deaths were reported, whereas in 2022 the incidence increased to 662,301 new cases with 348,874 deaths. In Indonesia, cervical cancer remains the second most common cancer among women after breast cancer. The number of newly diagnosed cases increased slightly from 36,633 cases in 2020 to 36,964 cases in 2022, while cervical cancer-related deaths were reported at 23,451 cases in 2020 and 20,708 cases in 2022. These findings indicate that cervical cancer continues to impose a substantial health burden and highlights the urgent need for more effective preventive and therapeutic strategies (Sung et al., 2021; Ferlay et al., 2021; Ferlay et al., 2024; Singh et al., 2023).

The prognosis and therapeutic outcomes of cervical cancer are strongly influenced by the stage of disease at diagnosis. Patients diagnosed with early-stage cervical cancer have a five-year survival rate exceeding 90%, whereas those diagnosed with advanced or metastatic disease have survival rates below 20%. Standard treatment for early-stage and locally advanced cervical cancer includes radical hysterectomy or radical trachelectomy combined with pelvic lymphadenectomy, concurrent chemoradiotherapy, or both. In contrast, treatment for metastatic cervical cancer primarily relies on systemic therapy. Despite advances in therapeutic approaches, the prognosis of advanced cervical cancer remains poor due to limited treatment efficacy and considerable adverse effects, emphasizing the necessity for novel therapeutic agents (Chou et al., 2022; Restaino et al., 2024; Tarney et al., 2025; Amirulah et al., 2026).

Current treatment modalities, including chemotherapy, radiotherapy, and immunotherapy, are also associated with several limitations, particularly the development of therapeutic resistance.

Treatment resistance contributes to disease recurrence, metastasis, and poor clinical outcomes. One of the principal mechanisms underlying therapeutic resistance is the ability of cancer cells to evade apoptosis, a programmed cell death process responsible for maintaining tissue homeostasis by eliminating damaged or abnormal cells (Fitriatuzzakiyyah et al., 2017; Sains et al., 2024; Boyina et al., 2023; Ojha et al., 2022).

Among the key regulators of apoptosis, B-cell lymphoma-2 (Bcl-2) is recognized as a major anti-apoptotic protein. Overexpression of Bcl-2 prevents mitochondrial outer membrane permeabilization and inhibits cytochrome c release, thereby suppressing apoptosis and promoting cell survival. Genetic alterations or increased expression of Bcl-2 in cancer cells enhance resistance to anticancer therapies, facilitate tumor progression and metastasis, and contribute to treatment failure. Consequently, targeting the Bcl-2 signaling pathway has emerged as a promising strategy for improving the effectiveness of cancer therapy (Ekundina & Omon, 2024).

To overcome treatment resistance and reduce disease recurrence and metastasis, recent therapeutic strategies have focused on combination therapies and the development of agents targeting alternative molecular pathways, including apoptosis. Natural products have attracted considerable attention as potential sources of novel anticancer compounds due to their diverse bioactive constituents and relatively low toxicity. Among these, durian peel (*Durio zibethinus* Murr.), an abundant agricultural by-product, has shown promising pharmacological potential. However, existing studies have mainly investigated its antioxidant and general cytotoxic activities, whereas its molecular mechanism, particularly its role in regulating the Bcl-2-mediated apoptotic pathway in cervical cancer, remains largely unexplored (Prasetyo et al., 2021).

Durian peel contains several biologically active flavonoids, primarily flavanones, flavonols, and flavones. Among these compounds, flavones exhibit stronger anticancer activity owing to their higher lipophilicity, which facilitates interaction with intracellular molecular targets. The major flavones identified in durian peel include apigenin and luteolin. Apigenin has been demonstrated to inhibit cancer progression by inducing apoptosis and autophagy, arresting the cell cycle, suppressing cell migration and invasion, and modulating immune responses. Likewise, luteolin promotes apoptosis by upregulating the expression of pro-apoptotic genes such as APAF1, BAX, BAD, BID, BOK, BAK1, TRADD, FADD, FAS, and caspases-3 and -9, while simultaneously downregulating anti-apoptotic genes including Bcl-2, Bcl-xL, and MCL-1. These findings suggest that flavonoids derived from durian peel possess considerable potential as apoptosis-inducing agents for cancer therapy (Khaksar et al., 2024; Raina et al., 2021; Tuli et al., 2022).

Despite these promising findings, evidence regarding the interaction of durian peel bioactive compounds with the Bcl-2 signaling pathway in cervical cancer remains scarce. This research gap provides an opportunity to investigate durian peel as a potential natural source of Bcl-2 inhibitors capable of enhancing the sensitivity of cervical cancer cells to therapy. Therefore, the present study aims to investigate the inhibitory activity of bioactive compounds from durian peel (*Durio zibethinus* Murr.) against the Bcl-2 pathway in cervical cancer. The findings are expected to provide a scientific basis for the development of natural product-based adjuvant therapies to overcome chemotherapy resistance and improve the clinical management of cervical cancer in the future.

RESEARCH METHODS

Study Design

This experimental laboratory study was conducted to evaluate the apoptotic activity of bioactive compounds extracted from durian peel (*Durio zibethinus* Murr.) against cervical cancer through inhibition of the B-cell lymphoma-2 (Bcl-2) pathway. The study consisted of phytochemical extraction, compound identification using Gas Chromatography–Mass Spectrometry (GC–MS), cytotoxicity assessment using the MTT assay, and immunofluorescence analysis to determine Bcl-2 expression in HeLa cervical cancer cells.

Materials

Fresh durian peels were collected, cleaned, dried, and processed into simplicia prior to extraction. Chemicals used included 96% ethanol, methanol, *n*-hexane, ethyl acetate, distilled water, chloroform, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), nitric acid (HNO_3), glacial acetic acid, magnesium powder, ferric chloride ($FeCl_3$ 1%), cerium sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and phytochemical reagents (Mayer, Wagner, and Dragendorff reagents). The equipment used for extraction included dark glass bottles (2.5 L), rotary evaporator, vacuum filtration apparatus, Whatman No. 40 filter paper, separatory funnels, volumetric flasks, beakers, test tubes, pipettes, hot plates, measuring cylinders, glass rods, and analytical balances. For cell culture experiments, HeLa cervical cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and maintained under standard cell culture conditions (37°C, 5% CO_2). Additional materials included phosphate-buffered saline (PBS), 96-well culture plates, T-75 culture flasks, sterile micropipette tips, centrifuge tubes, MTT reagent, dimethyl sulfoxide (DMSO) or formazan solvent, primary anti-Bcl-2 antibody, fluorescein isothiocyanate (FITC)-conjugated secondary antibody, and 4′,6-diamidino-2-phenylindole (DAPI) for nuclear staining.

Preparation of Durian Peel Extract

Approximately 2,500 g of fresh durian peel was cut into small pieces, dried under shade conditions, and ground into powder. The powdered simplicia was macerated with 96% ethanol at a ratio of 1:10 (w/v) for 72 hours with occasional stirring and protected from direct light. The extract was filtered using vacuum filtration with Whatman No. 40 filter paper. The filtrate was concentrated using a rotary evaporator at 55°C under 80 mbar pressure until a viscous crude extract was obtained. The extract was stored at 4°C until further analysis.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical constituents of the ethanol extract were identified using Gas Chromatography–Mass Spectrometry (GC–MS). The concentrated extract was injected into the GC injector, vaporized, and transported through the chromatographic column by an inert carrier gas. Individual compounds were separated according to their physicochemical properties and subsequently ionized in the mass spectrometer by electron impact ionization. The generated mass spectra were compared with the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) library for compound identification.

Cell Culture and MTT Cytotoxicity Assay

Human cervical cancer HeLa cells were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated for 24 hours to allow cell attachment. Cells were then washed twice with serum-free DMEM and serum-starved for 1 hour. Following starvation, cells were treated with different concentrations of durian peel ethanol extract (100, 150, 200, 250, and 300 µg/mL) for 24 hours. Untreated cells served as the negative control. After treatment, the culture medium was replaced with 100 µL DMEM containing MTT reagent, followed by incubation for 4 hours at 37°C in a humidified 5% CO_2 incubator. The medium was discarded, and the cells were washed with 200 µL PBS. Formazan crystals produced by viable cells were dissolved using 100 µL DMSO (or appropriate solvent) and mixed thoroughly by repeated pipetting. Cell viability was determined by measuring absorbance at 540 nm using a microplate reader. Cell viability (%) was calculated relative to the untreated control group, and the inhibitory concentration (IC_{50}) was determined from the dose-response curve.

Immunofluorescence Analysis of Bcl-2 Expression

HeLa cells were cultured on sterile glass coverslips placed in culture dishes and incubated for 24 hours to allow cell attachment. Cells were subsequently treated with the selected concentrations of durian peel extract for an additional 24 hours. Following treatment, cells were washed with phosphate-buffered saline (PBS) and fixed on microscope slides. The fixed cells were incubated with a primary anti-Bcl-2 antibody, followed by a fluorescein isothiocyanate (FITC)-conjugated secondary antibody for detection of Bcl-2 protein expression. Cell nuclei were counterstained with 4′,6-diamidino-2-

phenylindole (DAPI). Fluorescence images were captured using a fluorescence microscope. Green fluorescence represented Bcl-2-positive cells, while decreased green fluorescence intensity indicated reduced Bcl-2 expression and increased apoptotic activity following treatment with the durian peel extract.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro–Wilk test. Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A p-value of < 0.05 was considered statistically significant. The half-maximal inhibitory concentration (IC_{50}) was calculated by nonlinear regression analysis using GraphPad Prism version 10.0.

RESULTS AND DISCUSSION

Result

Results of simplisia and organoleptic parameters of extracts

Table 1. Results of simplisia and organoleptic parameters of extracts

Simplisia Parameter	Organoleptic of Extract
Durian peel moisture content = 8.7%	96% ethanol extract of durian peel powder with maceration method is thick, brown in color, has no taste, has a distinctive odor.

Table 2. Extract rendemen maseration method results

Weight of Extracted Powder	Total Weight Extract	Rendemen
2526.4 gram	206.7 gram	12.22%

Table 3. Phytochemical results

Parameter	Reaction	Observation
Alkaloid	+	Orange color
Flavonoid	+	Reddish orange color
Saponin	+	Foam
Tanin	+	Blackish-green color
Triterpenoid	+	Reddish-brown color

Results of Gas Chromatography and Mass Spectroscopy (GC-MS) GC-MS data showed that more than 93 bioactive compounds were obtained with RT and area values divided into 31 groups ranging from 3.059 to 42.824 for RT and 0.37 to 15.91% for abundance area. chromatogram of GC-MS analysis results of durian fruit peel can be seen in Figure 4.below.

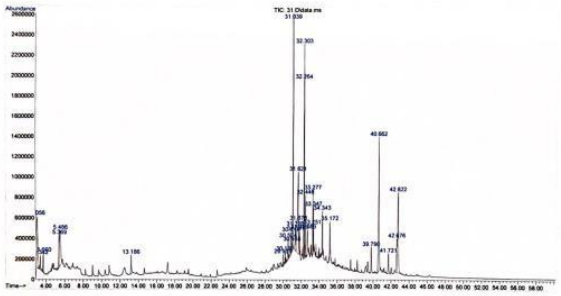


Figure 4. Chromatogram of the results of GCMS analysis of durian fruit peel. Results of in vitro analysis with durian peel extract treatment

Live and dead cells are morphologically different. (Figure 5 (A)) shows HeLa cells before treatment which are round and surrounded by a clear cell membrane. After treatment, the cancer cells undergo morphological changes, becoming non-spherical (Figure 5 (B)), and the formation of formazan crystals after MTT compound treatments (Figure 5 (C)). The cytotoxic activity of each treatment was evaluated based on the IC50 value. The IC50 value was obtained by plotting the log concentration of the treatment versus the % live cells.

A B C

Figure 5. (A) HeLa cells before treatment, (B). Cell death after treatment, (C) Formazan crystal formation

Table 7. Cytotoxic Test Results

Extract Name	Test Cells	*IC50* (µg/mL)	Activity
Durian peel	HeLa Cells	5.92	Active
Doxorubicin		1.59*	Highly active

IC50 activity is highly active = 5 µg/ml; active = 5-10 µg/ml; moderate = 11-30 µg/ml; inactive = >30 µg/ml.

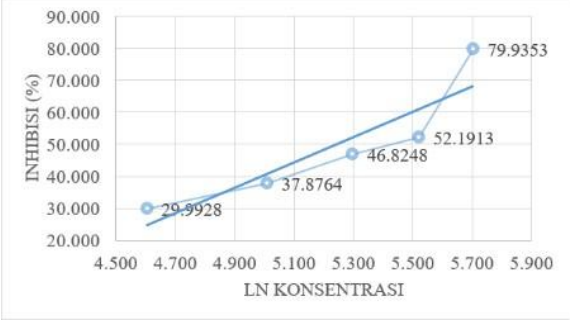
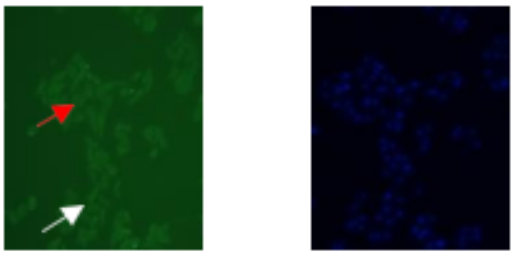


Figure 6. Inhibition rate in HeLa cells with durian peel treatment Results of Apoptosis Analysis by Immunofluorescent

Durian peel has cytotoxicity on cervical cancer HeLa cells by inducing apoptosis and works in decreasing the expressionof anti-apoptotic Bcl-2 (Figure 7).



A B

Discussion

In silico

In silico test results show that apigenin ligand has a binding energy (ΔG) value of -6.01 kcal/mol with an inhibition constant of 39.4 μM, and an RMSD of 14,670 Å. While luteolin has a binding energy value of -5.55 kcal/mol with an inhibition constant of 86.02 μM, and an RMSD of 17.489 Å. In comparison, fluorouracil and doxorubicin ligands have equally good binding values, but from the results obtained from ProTox predictions, it was found that doxorubicin has a higher toxicity effect. The results of 3D visualization using the Discovery Studio application also showed the presence of residual amino acids that confirmed the location of the active side of the Bcl-2 protein. The conclusion of the in silico study is that of the four compounds, apigenin compound is better as a candidate for cervical cancer therapy that is directly focused on target cells. But these in silico results only assess the attachment of compounds to proteins and do not assess the impact of administering these compounds. So further research is needed such as in vitro which was also carried out in this research.

Gas Chromatography and Mass Spectroscopy (GC-MS)

Based on the data on the results of the analysis, it can be seen that the durian fruit peel contains a lot of bioactive compounds, which shows the great potential of durian fruit peel to be utilized as an alternative source of antioxidants. The content of bioactive compounds from durian fruit peels with the lowest percent area is a compound of nonanoic acid, 9- oxo-, methyl ester (C10H18O3) with a molecular weight of 186.25 g/mol and the highest percent area is gamma-sitosterol (C29H50O) with a molecular weight of 414.7 g/mol.

In vitro

IC50 calculation results showed that flavonoid compounds of durian peel extract are active as anticancer in vitro with a value of 44.14 ± 18.69. The percentage of survival decreased significantly $p<0.05$ at the highest concentration (300 μg/ml). While doxorubicin as a standard drug for cancer treatment showed very active activity, but has the disadvantage of very high carcinogenicity.

Immunofluorescent

The effect observed in HeLa cells with durian peel treatment can be explained by inhibiting the anti-apoptotic Bcl-2, thus stimulating apoptosis again. The results showed that durian peel treatment was associated with a dose-dependent decrease in Bcl-2 expression. Cervical cancer HeLa cells respond to compounds in durian peel by inactivating Bcl-2 and inducing apoptosis).

CONCLUSION

This study suggests that durian peel (Durio zibethinus Murray) possesses promising anticancer activity against cervical cancer HeLa cells through the induction of apoptosis involving suppression of the anti-apoptotic Bcl-2 pathway. The ethanol extract demonstrated significant cytotoxic effects, reduced cell viability, and decreased Bcl-2 expression in vitro. Furthermore, molecular docking analysis indicated favorable interactions of the major flavonoid constituents, apigenin and luteolin, with the Bcl-2 protein, supporting their potential role in apoptosis regulation. These findings indicate that durian peel-derived flavonoids may serve as potential candidates for the development of

complementary therapeutic agents in cervical cancer management. Future studies are recommended to investigate the molecular mechanisms underlying these effects in greater detail, evaluate their efficacy in animal models, and assess their safety and therapeutic potential through preclinical and clinical investigations.

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