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Research Article

Anticancer activity of the ethylacetate fraction of *Vernonia amygdalina* Delile towards overexpression of HER-2 breast cancer cell lines

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Abstract

Vernonia amygdalina Delile, coming from the Asteraceae tribe, contains active compounds that can treat breast cancer. This study examines the anticancer activity of *Vernonia amygdalina* Delile leaves, an active fraction of MCF-7/HER-2 breast cancer cells. Thin-layer chromatography determines the phytochemical screening. Cytotoxic and proliferation analyses were determined using the MTT method for the MCF-7/HER-2 breast cancer cell line. The cell cycle and apoptosis profiles were examined using flow cytometry. The results can be summarized in this study: *Vernonia amygdalina* Delile fraction contains a flavonoid with great potential for pharmacological activities, for instance, inhibiting the growth of cancer cells. The ethyl acetate fraction was more potent and cytotoxic on MCF-7/HER-2 cells (IC_{50} = 66 µg/mL) than extract ethanol (IC_{50} = 130 µg/mL). The ethylacetate fraction of *Vernonia amygdalina* Delile has been proven to inhibit cell proliferation by decreasing cell viability with an IC_{50} of 66 µg/mL concentration incubated for 24, 48, and 72 hrs with cell viability values of 64.46%, 61.67%, and 53.89%, respectively, compared to the ethanol extract IC_{50} of 130 µg/mL concentration with cell viability values of 56.0%, 50.19%, and 58.67%, respectively, which induced apoptosis and inhibited the cell cycle in the G_2-M phase. To conclude, the ethyl acetate fraction of *Vernonia amygdalina* Delile can be used as an anticancer against the MCF-7/HER-2 cell line with an IC_{50} of 66 µg/mL, inhibiting cell proliferation and the cell cycle and inducing apoptosis activity in the MCF-7/HER-2 cell.

Keywords

ethylacetate, *Vernonia amygdalina* Delile, breast cancer, overexpression, HER-2

Introduction

The uncontrolled process of cell development is known as cancer, initiated by cell expansion that can scatter throughout the body's tissue, called metastasis. Cancer's continual growth results in an imbalance between live and dead cells (Burhan et al. 2021). Apoptosis and proliferation have pivotal roles in regulating the system in individuals. It is essential to maintain the balance of apoptosis and proliferation in cells. If there is uncontrolled apoptosis, there will be decreased organ system function, resulting in diseases. The uncontrolled proliferation will form a tumor, resulting in cancer (Safriana et al. 2018). Cell abnormalities happen because the cells grow uncontrollably, which is called cancer. Many women fear breast cancer because of the uncontrolled growth of the cells. Cancer can invade breast tissue, forming breast cancer. Many factors can cause breast cancer, for instance, the history of reproductivity, lack of physical activity, free radicals, the presence of mutations in genes, and radiation (Hasibuan et al. 2020a).

Breast cancer has become the fifth-ranking cancer among women in the world and has become the most common cancer among women as it is causing cancer-related deaths in women. An estimated 324.000 women passed away from breast cancer, which accounts for 14.3% of the death rate from breast cancer. Malignant tumors around women's breast area grow in the breast tissue and spread to another body part, which is known as metastasis (Hasibuan et al. 2021).

Apoptosis is an important mechanism to prevent the proliferation of cells that experience DNA (deoxyribonucleic acid) damage. Apoptosis is one of the checkpoints in the cell cycle. Controlling the cell cycle is very crucial. The uncontrolled cell cycle can cause damaged cells to continue to grow and divide into cancer cells. In other words, apoptosis is a process of natural cell death to preserve cells' integrity, depending on the cell's biochemical mechanism (Hasibuan et al. 2020b).

There are several ways to treat cancer; for instance, using doxorubicin has proven to be effective for cancer treatment. The utilization of doxorubicin has been limited because of cardiotoxicity as an adverse effect. Another study reported that 2.2% of patients who received doxorubicin had heart failure. The mechanism of doxorubicin is to form superoxide and free radicals (Syahputra et al. 2021). Since doxorubicin causes cardiotoxicity, herbal medicine has become another alternative with fewer adverse effects (Dalimunthe et al. 2024). Indonesia has many kinds of plants with tremendous anticancer functions, one of which is *Vernonia amygdalina* Delile, an African original plant from the Asteraceae tribe. They can be found in tropical areas such as Indonesia and have been widely utilized as a traditional medicine from the *Vernonia* genus (Syahputra et al. 2020).

Vernonia amygdalina Delile (African leaves) plants are proven to have tremendous potential as an anticancer because they contain many flavonoid compounds, including tannin, saponins, alkaloids, terpenes, steroid glycosides, sesquiterpene lactones, and triterpenoids (Bestari 2021; Prananda et al. 2023). *Vernonia amygdalina* Delile plant cannot only be utilized as an anticancer but also can be utilized for several treatments, including diabetes treatment, headache, toothache, antimalarial, antibacterial, nephroprotection, antioxidant, analgesic, antioxidant, and hepatoprotection (Harahap et al. 2021a), and based on a previous study proven by Burhan in 2021, African leaves contain flavonoids and bioactive compounds that have potential as an anticancer. Flavonoid compounds not only have great potential as a breast anticancer by inhibiting the activity of DNA topoisomerase I/II, resulting in the induction of cell apoptosis (Mahmod et al. 2024). African leaves contain another active compound utilized as an anticancer, cardiac glycosides, which inhibit the activity of cancer cells. The content of cardiac glycosides is unsaturated α and β lactone rings, affecting Na^+ and K^+ pumps (Hasibuan et al. 2020a).

The cytotoxic cell assay uses IC_{50} (half-maximal inhibitory concentration) values to represent a concentration of extract that could inhibit 50% of cancer cells, resulting in 50% inhibition of cell proliferation and indicating drug toxicity towards MCF-7/HER-2 (Michigan Cancer Foundation-7/Human Epidermal Growth Receptor 2) cells. HER-2 has emerged as a promising target for cancer therapy (Satria et al. 2024). The purpose was to examine the growth of the cell and obtain the cycle phase, which experienced growth during the G_2 phase, which is affected by carcinogenic substances resulting in the damage of DNA. The unrepairable DNA triggers apoptosis. The value of apoptosis and the cycle phase can be evaluated using flow cytometry (Hasibuan and Fachrunisa 2019).

Material and method

Plants and materials

Fresh leaves of *Vernonia amygdalina* Delile were collected in 2022 in Medan, Indonesia. African leaves used in this research were confirmed at the Indonesian Institute of Sciences, Pusat Penelitian Biologi, Cibinong, Indonesia. Annexin-V (BioLegend), dimethyl sulfoxide (Merck), ethanol (Merck), ethylacetate (Merck), n-hexane (Merck), propidium iodide (BioLegend), distilled water, and [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma) were the substances used in the research.

Ethical approval

This study has gained ethical approval No. 1181/KEPK/2022 from the research ethics committee of Universitas Sumatera Utara.

Crude extract and fraction preparation

Reflux was the method being used to make crude extract. The leaves were shade-dried, pulverized, and stored at room temperature, and then the reflux apparatus was

assembled. 100 g of powdered *Vernonia amygdalina* Delile was extracted by 1400 mL of absolute ethanol for 3–4 hrs. The suspension was filtered and concentrated to dryness to get the crude extract using a rotary evaporator. The extractions were made in triplicate with solvent replacement and viscous extract as the result of this process.

The viscous extract was fractionated into n-hexane, ethyl acetate, and water fractions. This fractionation was done using the liquid-liquid extraction method. The viscous crude extract (100 g) was suspended in 500 ml of distilled water and fractionated using organic solvents with increasing polarity (N-hexane, ethyl acetate, and water). Each fraction from the ethanol extracts was tested as an anticancer using MCF-7/HER-2 cell lines (Alara et al. 2019).

Phytochemical analysis

The thin-layer chromatography method analyzes the bioactive components contained in African leaves. A plate coated with silica gel 60 GF254 (Merck, Germany) is used as the stationary phase; meanwhile, the mobile phase is used depending on the examination. After the elucidation process, the plate was removed and dried; then, it was sprayed with an appropriate spectrophotometer, heated in an oven at 110 °C for 5 minutes, and examined for color change (Wagner and Bladt 1996).

a. **Flavonoid** identification used mobile phase ethyl acetate-methanol-water (100:13.5:10). Then the results were stained with an $AlCl_3$ 10% reagent. The yellow, green, or blue fluorescence reveals a positive reaction.
b. **Alkaloid** identification used mobile-phase chloroform-methanol-ammonia (85:15:1). Dragendorff's reagent was then used as a stain. The formation of orange-brown zones reveals a positive reaction.
c. **Steroids/Triterpens** identification used mobile phase n hexanes: ethyl acetate (8:2). The results were stained with the Lieberman-Bouchard reagent. The formation of red-violet zones reveals a positive reaction.
d. **Glycoside** identification used mobile-phase ethyl acetate-methanol-water (8:1:1). The results were stained with a 50% sulfuric acid reagent. A positive reaction is revealed by forming violet-red, yellow-brown, dark blue-black, or bright blue zones.
e. **Tannin** identification used mobile phase chloroform-acetate acid-n butanol-water (5:2:2:1). The results were stained with a $FeCl_3$ 1% reagent. The formation of gray and brown zones reveals a positive reaction.
f. **Saponin** identification used mobile phase chloroform-acetate acid-methanol-water (11:6:2:1). The results were stained with an anisaldehyde-sulfuric acid reagent. A positive reaction is revealed by forming blue-violet and occasionally red or yellow-brown zones.

Cell line and culture medium

MCF-7/HER-2 cells are the luminal breast cancer subtype purchased from the Laboratory of Parasitology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, in Yogyakarta. MCF-7/HER-2 were inoculated in Dulbecco's Modified Eagle Medium (DMEM) enhanced with 10% FBS (fetal bovine serum), 1% penicillin/streptomycin (Gibco), and 0.5% fungizone (Gibco) at 37 °C with 5% CO_2 (Bashari et al. 2022).

Cytotoxic assay

MCF-7/HER-2 cells (5000/well) seed in a 96-well plate, followed by incubation at 37 °C in a 5% CO_2 incubator for 24 hrs. The plate contained medium, and incubated cells were discarded and re-incubated by administering ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction with different concentrations. The process continues with overnight incubation at 37 °C in a 5% CO_2 incubator. Following incubation, 0.5 mg/mL MTT was added to each well, and the plate was incubated for 4 hrs at 37 °C in 5% CO_2. The excess MTT was aspirated, and the formazan crystals formed were dissolved by administering 10% SDS (Sigma) in 0.01N HCl (Merck). The incubation of cells was done overnight at room temperature in a dimly lit place. Eventually, the absorbance was recorded at λ 595 nm wavelength using a microplate reader. The data absorbed from each well were converted to viability percentage cells (Foo et al. 2014).

Cell viability and cell proliferation assays

For the MTT assay, the cells were incubated in 96-well plates at two cells/well density. The cells with the same density rate were made into three 96-well microplates, followed by incubation at 37 °C in a 5% CO_2 incubator overnight. The plate contained medium, and incubated cells were discarded and then re-incubated with ethanol extract and ethylacetate fraction with different concentrations in a 5% CO_2 incubator at 37 °C for 24, 48, and 72 hrs after the given extract and fraction. The cells were allowed to attach and proliferate during the desired incubation time. Following incubation, 0.5 mg/mL MTT was administered in each well, and the plate was incubated for 4 hrs at 37 °C. After four hours, the cells were given 10% SDS (Sigma) in 0.01N HCl (Merck) to dissolve the formazan crystals. The cells were incubated for 24 hrs at room temperature in a dimly lit place. Eventually, the absorbance was recorded at λ 595 nm wavelength using a microplate reader. The data absorbed from each well were converted to viability percentage cells (Wei et al. 2017).

Cell preparation for flow cytometry analysis

The MCF-7/HER-2 cells (500.000 cells/mL) were inoculated in a 6-well plate following incubation for 24 hrs at 37 °C in 5% CO_2. The conical tube was used to put the media in

and rinsed with PBS (Phosphate Buffer of Standard), then the cells were given ethyl acetate and doxorubicin with different concentrations following incubation for 24 hrs at 37 °C in 5% CO_2. After administering 0.025% trypsin to the cells, they were collected into labeled conical tubes. PBS was used to rinse and was collected in the labeled conical tube. The media was centrifuged at 2.500 rpm for 5 minutes, and the sediment was compiled, while the supernatant was discarded (Hasibuan and Fachrunisa 2019).

Cell cycle analysis

DNA flow cytometry analysis was used to examine all phases of the cells. The sediment was added to the ethanol for 4 hrs, which is called the cell fixation process. Before the supernatant was discarded, PBS was added and centrifuged at 20,000 rpm for 3 minutes, then RNAse and PI were re-suspended. Then, the mixture was incubated for 30 minutes at 37 °C (Hasibuan and Fachrunisa 2019). Modfit software was utilized to examine total percentages for all cell-cycle phases (Kwan et al. 2016).

Apoptosis analysis

Annexin V-FITC (V fluorescein isothiocyante) was greatly utilized to quantify and identify the apoptosis of the cell. Briefly, PI (Propidium iodide) and Annexin V-FITC were added to the cells following incubation for approximately 15 minutes in the dark at room temperature. Flow cytometry measures the intensity of FITC/PI fluorescence (Yedjou et al. 2013).

Result and discussion

Phytochemical screening

As can be seen in Table 1, the phytochemical screening result of the crude ethanol extract of *Vernonia amygdalina* using thin-layer chromatography showed the presence of several of the bioactive components tested, including flavonoids, tannins, saponins, glycosides, and steroids and triterpenes (Harahap et al. 2021b). N-hexane and ethyl acetate are the solvents used in this research for liquid-liquid extraction. N-hexanes fraction, ethylacetate fraction, and water fraction were fractionated from ethanol extract. The N-hexane fraction contained bioactive components such as flavonoids, tannins, glycosides, and steroids and triterpenes. Ethylacetate fractions contained flavonoids, tannins, glycosides, saponins, steroids, and triterpenes. The water fraction contained bioactive compounds such as flavonoids, alkaloids, saponins, and glycosides. These bioactive compounds have a pivotal role in the pharmacological activities of plants (Alebiosu and Yusuf 2015; Hasibuan et al. 2021).

Table 1. Phytochemical screenings of *Vernonia amygdalina* Delile.

Phytochemicals	Ethanol extract	N-Hexane fraction	Ethylacetate fraction	Water fraction
Alkaloids	-	-	-	+
Flavonoids	+	+	+	+
Saponins	+	-	+	+
Tannins	+	+	+	-
Glycosides	+	+	+	+
Steroids/ Triterpenoids	+	+	+	-

Cytotoxic activity of *Vernonia amygdalina* Delile

The MTT technique (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was used in determining the inhibition value of a 50% death cell, which is the parameter in this study (Juwitaningsih et al. 2022). The mitochondrial activity of cells is the ground principle in this research. The result found that the ethyl acetate fraction IC_{50} value is 66 µg/mL, which is classified as a strong cytotoxic effect compared to other extracts and fractions (Table 2). According to the IC_{50} value classification, the activity of anticancer is divided into several groups: IC_{50} value ≤ 10 µg/mL is classified as highly active; IC_{50}: 20–100 µg/mL is classified as active; IC_{50}: 100–500 µg/mL is classified as moderate cytotoxic effect (Fithrotunnisa et al. 2020). Based on the screening result, it was found that the ethyl acetate fraction contained secondary metabolites, which were classified as anticancer substances, for instance, flavonoids. Flavonoid compounds have not only great potential as a breast anticancer by inhibiting the activity of DNA topoisomerase I/II, resulting in the induction of apoptosis in cells, but also have great potential in decreasing and inhibiting the activity of endonuclease and the expression of Bcl-2 and Bcl-XL genes because of the presence of flavonoid bioactive compounds (Hasibuan and Fachrunisa 2019; Hasibuan et al. 2021).

Table 2. IC_{50} of *Vernonia amygdalina* Del extracts and fractions.

Treatments	Concentrations (µg/mL)	Vero	SI
Ethanol extract	130 µg/mL ± 0.127	-	-
Ethylacetate fraction	66 µg/mL ± 0.175	-	-
N- Hexane fraction	168 µg/mL ± 0.137	-	-
Doxorubicin	1.8 µg/mL ± 0.098	-	-

Cell viability and cell proliferation assays of *Vernonia amygdalina* Delile

As a basis for further characterization of *Vernonia amygdalina* Delile of cullar response in MCF-7/HER-2, the ethanolic extract and ethylacetate fraction were examined for inhibiting cell proliferation by using the MTT assay (Hasibuan et al. 2020a). The result indicated that treating MCF-7/HER-2 cells with different ethylacetate fraction concentrations and ethanol extracts induced cell proliferation rates.

The bar chart (Fig. 1) shows that the viability cells of MCF-7/HER-2 treated with an ethylacetate fraction concentration of 66 g/mL showed the best result in inhibiting cell proliferation. The percentage of cell viability increased from 64.46% within 24 hrs of incubation to 61.67% within 48 hrs, a decrease in the percentage of cell viability to 53.89% within 72 hrs of incubation, which means the cells experience saturation. As it also shows no increase in cell growth until 72 hrs of incubation, this is probably due to cell death. This death occurs through a cell cycle arrest

mechanism, so that the ability of MCF-7/HER-2 cells to proliferate decreases. It proves that the ethylacetate fraction of African leaves has the potential to inhibit cell proliferation (Wong et al. 2013; Burhan et al. 2021).

The bar chart (Fig. 2) shows that the viability cells of MCF-7/HER-2 treated with an ethyl acetate fraction concentration of 66 g/mL showed the best result in inhibiting cell proliferation. The percentage of cell viability decreased from 56.0% within 24 hrs of incubation to 50.19% within 48 hrs. There was a decrease in the percentage of cell viability to 58.67% within 72 hrs of incubation, which means the cells experienced saturation at 48 hrs. The ethanol extract from African leaves has the potential to inhibit cell proliferation through dose-dependent concentration (Hasibuan and Fachrunisa 2019; Hasibuan et al. 2021).

Based on the bar chart (Fig. 3), doxorubicin has a tremendous potential to inhibit cell proliferation through time-dependent concentrations. Doxorubicin is a chemotherapeutic agent for various types of cancer, such as breast cancer. The mechanism of action of doxorubicin is by inhibiting the topoisomerase II enzyme; this enzyme works by binding to cancer cell DNA and blocking enzymes necessary for cancer cell growth, such as the topoisomerase II enzyme (Hasibuan and Fachrunisa 2019).

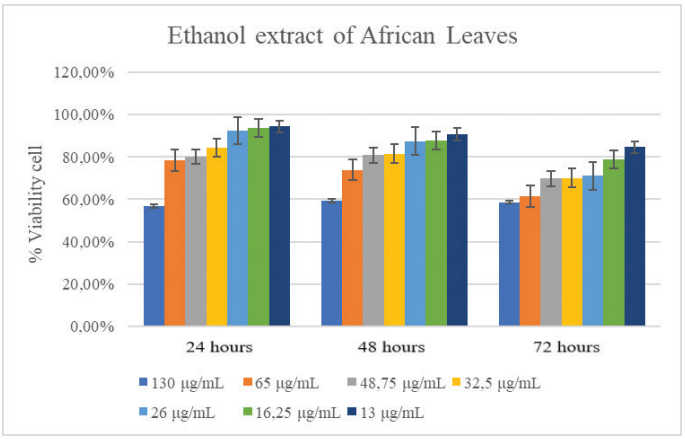

Figure 1. Proliferation profiles of ethanol extract with different concentrations.

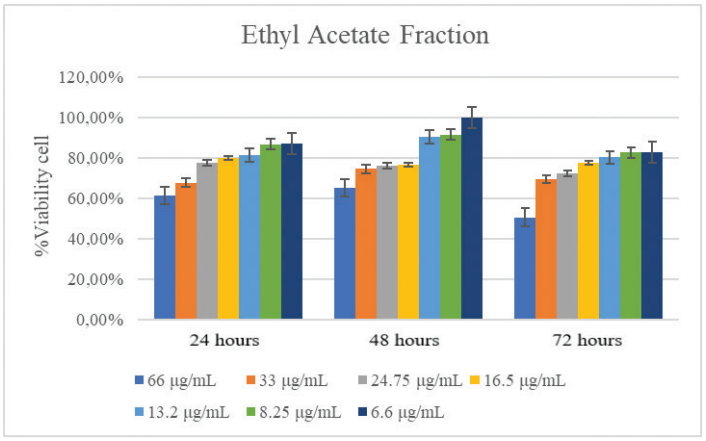

Figure 2. Proliferation profiles of ethylacetate with different concentrations.

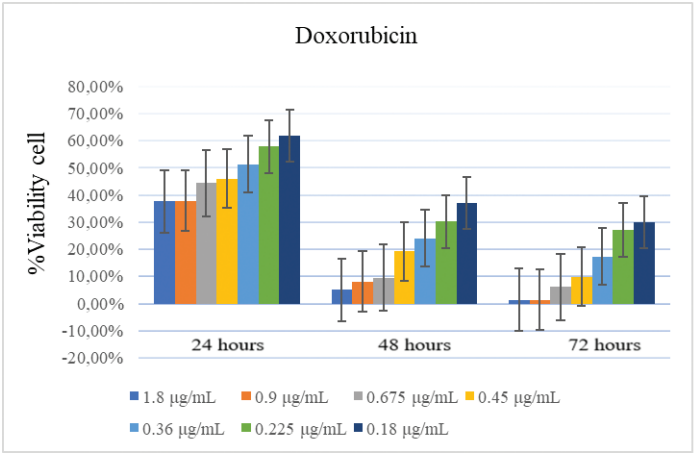

Figure 3. Proliferation profiles of doxorubicin with different concentrations.

Cell cycle inhibition analysis of *Vernonia amygdalina* Delile

MCF-7/HER-2 was incubated with 33.0 µg/mL ethyl acetate to examine the effects on cell cycle inhibition by flow cytometry. The result indicated for the G_0-G_1 phase of cell control was 38.3% (Table 3 and Fig. 4). After 24 hrs of treatment with 33.0 µg/mL ethylacetate at 38.6%, it was revealed that ethyl acetate induced the G_0-G_1 phase. In phases S and G_2-M, cells were treated with 33.0 µg/mL ethylacetate with 20.9% and 17.3. It can be concluded that the ethylacetate fraction has tremendous potential to inhibit the cell cycle of MCF-7/HER-2 compared to the cell control in phase G_2-M with 15.7%. Inducing apoptosis and arresting the cell cycle in the G_0-G_1, S, and G_2-M phases are the best ways to inhibit the growth of cancer cells, as many anticancer agents do. Ethylacetate in the S phase and DNA repair in the G_2-M phase of cycle cells trigger the death cell mechanism (apoptosis), which is crucial during cell growth regulation (Hasibuan and Fachrunisa 2019; Simanullang et al. 2022). To be more specific, the most important target to be utilized as an anticancer therapy is the phase during the G_2-M checkpoint in the cell cycle, resulting not only in the prevention of cell DNA damage by letting it enter the mitosis but also giving the space and time for repairing the DNA damage, which can enhance the cytotoxic chemotherapy effect (Kwan et al. 2016; Hasibuan et al. 2020b). Flavonoid compounds have great potential as breast anticancers by inhibiting DNA topoisomerase I/II activity, resulting in cell apoptosis (Mahmod and Talib 2021). The ethyl acetate fraction can enhance the accumulation of G_2-M cell phases in the cell cycle, indicating damaged DNA repair. Not only are flavonoid compounds known to have anticancer potential, but saponin compounds contained in African leaves also have the potential to be anticancer. Saponins act as an anticancer by inducing cell cycle arrest in the G1 phase (Burhan et al. 2021; Hudan and Patricia 2022).

Table 3. Cell cycle inhibition analysis of the *Vernonia amygdalina* ethyl acetate fraction.

Treatments	Concentrations (µg/mL)	Phases		
		G_0-G_1	S	G_2-M
Cell Control	-	38.3	25.5	15.7
Ethylacetate Fraction	33.0	38.6	20.9	17.3
Doxorubicin	0.9	18.9	36.7	26.1

Apoptosis analysis of *Vernonia amygdalina* Delile

The flow cytometry method was used to determine apoptosis on purpose to calculate the number of living cells, cell necrosis, and apoptosis within a short period. MCF-7/HER-2 was given Annexin V to bind phosphatidylserine found in the cell plasma membrane during fluorescence apoptosis (Hasibuan and Fachrunisa 2019). As seen in Table 4 and Fig. 5, MCF-7/HER-2 cells were administered with ethylacetate fraction 33.0 µg/mL, resulting in early apoptosis of 21.8%, late apoptosis of 11.6%, and final necrosis of 26.1% compared to cell control, which was not treated with ethylacetate in the early apoptosis with 7.1%, which is lower than ethyl acetate. It indicates that the ethyl acetate fraction can be used to promote apoptosis. The ethylacetate fraction of African leaves has the potential to induce apoptosis. Based on the phytochemical screening displayed, the ethyl acetate fraction contained tannins, bioactive compounds that

Table 4. Apoptosis analysis of *Vernonia amygdalina* Del ethylacetate fraction.

Treatment	Concentration µg/mL	LL	LR	UR	UL
Control Cell	-	87.7	7.1	2.8	2.9
Ethyl Acetate Fraction	33.0 µg/mL	43.0	21.8	11.6	26.1
Doxorubicin	0.9 µg/mL	43.0	51.7	4.6	1.0

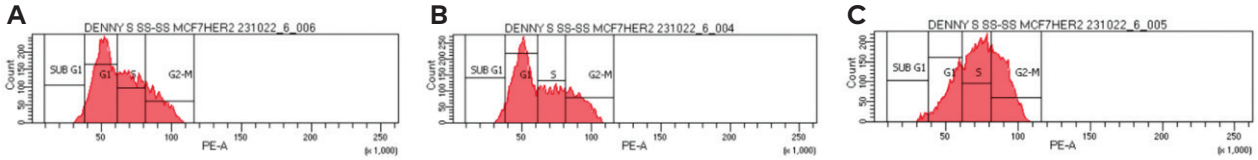

Figure 4. Cell cycle analysis of apoptosis of MCF-7/HER-2 cells: **A.** Control cell; **B.** Treatment with 33.0 µg/mL ethylacetate fraction; **C.** Treatment with 0.9 µg/mL doxorubicin.

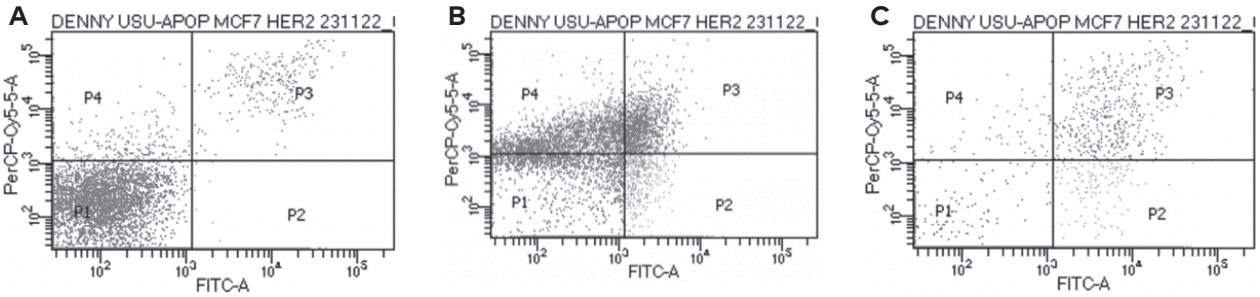

Figure 5. Flow cytometry analysis of apoptosis of MCF-7/HER-2 cells: **A.** Control cell; **B.** Treatment with 33.0 µg/mL ethylacetate fraction; **C.** Treatment with 0.9 µg/mL doxorubicin.

function as anticancer, initiating apoptosis pathways, and decreasing protein kinase activation (Okoduwa et al. 2020; Burhan et al. 2021).

Conclusion

In conclusion, it was found that the ethyl acetate fraction of African leaves has an immensity anticancer effect on MCF-7/HER-2 with an IC_{50} of 66 µg/mL. Therefore, it can also be utilized for breast cancer treatment by inhibiting proliferation, cell time-concentration-dependent inhibition of the cell cycle, and inducing the activity of apoptosis.

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References

Alara OR, Abdurahman NH, Ukaegbu CI, Kabbashi NA (2019) Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. Journal of Taibah University for Science 13(1): 414–422. https://doi.org/10.1080/16583655.2019.1582460

Alebiosu CO, Yusuf AJ (2015) Phytochemical screening, thin-layer chromatographic studies and UV analysis of extracts of *Citrullus lanatus*. JPCB 3(2): 218. [Available online at] http://www.jpcbs.info

Bashari M, Fadhil M, Aulia Y, Sari AK, Putri T, Qomarilla N, Atmaja H, Sudji IR, Ariyanto EF, Indrati AR, Rohmawaty E (2022) The ethyl acetate fraction of marine sponge stylissa carteri induces breast cancer cell death via upregulation of Mcl-1S: an in vitro study. Asian Pacific Journal of Cancer Prevention 23(5): 1653–1660. https://doi.org/10.31557/APJCP.2022.23.5.1653

Bestari R (2021) Senyawa fitokimia dan aktivitas farmakologis daun afrika (*Vernonia amygdalina* Del.) sebagai kandidat obat herbal. Jurnal Kedokteran STM (Sains Dan Teknologi Medik) 4(1): 63–74. https://doi.org/10.30743/stm.v4i1.135

Burhan A, Kamaruddin M, Ahmad R, Marzuki I, Misriyani (2022) Anticancer and cytotoxic potentials of *Vernonia amygdalina* Delile on WiDr cell lines. Phytopharmacology Research Journal 1(1): 1–7. https://ojs.prjn.org/index.php/prjn/article/view/6

Dalimunthe A, Satria D, Sitorus P, Harahap U, Angela IFD, Waruwu SB (2024) Cardioprotective effect of hydroalcohol extract of andaliman (*Zanthoxylum acanthopodium* DC.) fruits on doxorubicin-induced rats. Pharmaceuticals 17(3): 359. https://doi.org/10.3390/ph17030359

Fithrotunnisa Q, Arsianti A, Kurniawan G, Qorina F, Tejaputri NA, Azizah NN (2020) In vitro cytotoxicity of *Hibiscus sabdariffa* Linn extracts on A549 lung cancer cell line. Pharmacognosy Journal 12(1): 14–19. https://doi.org/10.5530/pj.2020.12.3

Foo JB, Yazan LS, Tor YS, Armania N, Ismail N, Imam MU, Yeap SK, Cheah YK, Abdullah R, Ismail M (2014) Induction of cell cycle arrest and apoptosis in caspase-3 deficient MCF-7 cells by *Dillenia suffruticosa* root extract via multiple signalling pathways. BMC Complementary and Alternative Medicine 14(1): 197. https://doi.org/10.1186/1472-6882-14-197

Harahap U, Dalimunthe A, Haro G, Syahputra RA, Widodo, Utomo DH, Satria D (2021a) In-silico analysis of cardiac glycosides from *Vernonia amygdalina* Delile. leaves as cardiotonic through inhibition of Na^+/K^+ ATPase ion transport. Rasayan Journal of Chemistry 14(01): 101–104. https://doi.org/10.31788/RJC.2021.1415800

Harahap U, Dalimunthe A, Hertiani T, Muhammad M, Nasri, Satria D (2021b) Antioxidant and antibacterial activities of ethanol extract of *Vernonia amygdalina* Delile. Leaves. AIP Conference Proceedings 2342(1): 080011. https://doi.org/10.1063/5.0045447

Hasibuan PAZ, Fachrunisa D (2019) Cell cycle inhibition and apoptotic induction of *Vernonia amygdalina* Del. leaves extract on MCF-7 cell line. Open Access Macedonian Journal of Medical Sciences 7(22): 3807–3810. https://doi.org/10.3889/oamjms.2019.509

Hasibuan PAZ, Harahap U, Sitorus P, Lubis MF, Satria D (2021) In-silico analysis of vernonioside d and vernonioside e from *Vernonia amygdalina* Delile. leaves as inhibitor of epidermal growth factor receptor (EGFR) and mammalian target of rapamycin (mTOR). Rasayan Journal of Chemistry 14(03): 1539–1543. https://doi.org/10.31788/RJC.2021.1436092

Hasibuan PAZ, Harahap U, Sitorus P, Satria D (2020a) The anticancer activities of *Vernonia amygdalina* Delile. leaves on 4T1 breast cancer cells through phosphoinositide 3-kinase (PI3K) pathway. Heliyon 6(7): e04449. https://doi.org/10.1016/j.heliyon.2020.e04449

Hasibuan PAZ, Munir D, Pertiwi D, Satria D, Lubis MF (2020b) Flavonoids constituent analysis and cell cycle inhibition activity of ethylacetate extract of *Vernonia amygdalina* Delile. leaves on lung cancer cell line. Rasayan Journal of Chemistry 13(04): 2577–2581. https://doi.org/10.31788/RJC.2020.1345625

Hudan SH, Praticia VM (2022) Telaah efek farmakologi daun afrika (*Vernonia amygdalina* Del) serta senyawa aktif di dalamnya. Jurnal Riset Farmasi 2(1): 9–14. https://doi.org/10.29313/jrf.v2i1.700

Juwitaningsih T, Roza D, Silaban S, Hermawati E, Windayani N (2022) Phytochemical screening, antibacterial, antioxidant, and anticancer activity of coffee parasite acetone extract (*Loranthus ferrugineus* Roxb). Pharmacia 69(4): 1041–1046. https://doi.org/10.3897/pharmacia.69.e91427

Kwan YP, Saito T, Ibrahim D, Al-Hassan FMS, Ein Oon C, Chen Y, Jothy SL, Kanwar JR, Sasidharan S (2015) Evaluation of the cytotoxicity, cell-cycle arrest, and apoptotic induction by *Euphorbia hirta* in MCF-7 breast cancer cells. Pharmaceutical Biology 54: 1223–1236. https://doi.org/10.3109/13880209.2015.1064451

Mahmod AI, Talib WH (2021) Anticancer activity of *Mandragora autumnalis*: an in vitro and in vivo study. Pharmacia 68(4): 827–835. https://doi.org/10.3897/pharmacia.68.e71695

Mahmod AI, Oqal M, Khalid AM, Afifi FU, Talib WH (2024) Phytochemical analysis, antioxidant, and antitumor activity of *Ligustrum ovalifolium* leaves grown in Jordan: an *in vitro* and *in vivo* study. Pharmacia 71: 1–10. https://doi.org/10.3897/pharmacia.71.e111517

Okoduwa SIR, Umar IA, James DB, Inuwa HM, Habila JD, Venditti A (2021) Bioguided fractionation of hypoglycaemic component in methanol extract of *Vernonia amygdalina*: an in vivo study. Natural Product Research 35(24): 5943–5947. https://doi.org/10.1080/14786419.2020.1805605

Prananda AT, Dalimunthe A, Harahap U, Syahputra RA, Nugraha SE, Situmorang PC, Fah YT, Adrian, Siahaan JM, Velaro AJ, Bilakaya B, Harahap MAY (2023) Phytochemical profiling and cardioprotective activity of *Vernonia amygdalina* ethanol extract (VAEE) against ISO-induced cardiotoxicity in rats. Pharmacia 70(3): 758–796. https://doi.org/10.3897/pharmacia.70.e112425

Safriana, Rosidah, Hasibuan PAZ, Satria D (2018) Cytoprotective effect of ethanol fraction of *Vernonia amygdalina* Delile leaves against the vero cells. Asian Journal of Pharmaceutical and Clinical Research 11(1): 214. https://doi.org/10.22159/ajpcr.2018.v11s1.26610

Satria D, Waruwu SB, Sholikhah EN, Mustofa, Satriyo PB, Wahyuningsih TD, Wiraswati HL, Damayanti E (2024) In-silico analysis of N-phenyl pyrazoline derivates as potential of human epidermal growth receptor-2 (HER-2) inhibitor using molecular docking and MD simulations. Rasayan Journal of Chemistry 17(2): 356–362. https://doi.org/10.31788/RJC.2024.1728795

Simanullang RH, Situmorang PC, Siahaan JM, Widjaja SS, Mutiara M (2022) Effects of *Zanthoxylum acanthopodium* on MMP-9 and GLUT-1 expression and histology changes in rats with cervical carcinoma. Pharmacia 69(4): 911–920. https://doi.org/10.3897/pharmacia.69.e89368

Syahputra RA, Harahap U, Dalimunthe A, Nasution P, Haro G, Widodo, Utomo DH, Satria D (2020) In-silico toxicity prediction of bioactive compounds of *Vernonia amygdalina* Delile. and digoxin. Rasayan Journal of Chemistry 13(02): 1220–1224. https://doi.org/10.31788/RJC.2020.1325638

Syahputra RA, Harahap U, Dalimunthe A, Pandapotan M, Satria D (2021) Protective effect of *Vernonia amygdalina* Delile against doxorubicin-induced cardiotoxicity. Heliyon 7(7): e07434. https://doi.org/10.1016/j.heliyon.2021.e07434

Wagner H, Bladt S (1996) Plant drug analysis. Springer, New York. https://doi.org/10.1007/978-3-642-00574-9

Wei ML, Duan P, Wang ZM, Ding M, Tu P (2017) High glucose and high insulin conditions promote MCF-7 cell proliferation and invasion by upregulating IRS1 and activating the Ras/Raf/ERK pathway. Molecular Medicine Reports 16(5): 6690–6696. https://doi.org/10.3892/mmr.2017.7420

Wong FC, Woo CC, Hsu A, Tan BKH (2013) The anti-cancer activities of *Vernonia amygdalina* extract in human breast cancer cell lines are mediated through caspase-dependent and p53-independent pathways. PLOS ONE 8(10): e78021. https://doi.org/10.1371/journal.pone.0078021

Yedjou CG, Izevbigie EB, Tchounwou PB (2013) *Vernonia amygdalina*-induced growth arrest and apoptosis of breast cancer (MCF-7) cells. Pharmacology & Pharmacy 4(1): 93–99. https://doi.org/10.4236/pp.2013.41013