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Africa leaf (*Vernonia amygdalina* Delille.) DCM extract synergistically supports growth suppression effect of doxorubicin on MCF7 and MCF7/HER2 with different effects on cell cycle progression and apoptosis evidence

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ABSTRACT

Africa leaf (*Vernonia amygdalina* Delille) has low cytotoxic activities against several cancer cells. We evaluated its combination treatment with doxorubicin against luminal breast cancer cells against MCF-7 and MCF-7/HER2 cells. The dried leaves were extracted to collect Hexane, Butanol, Dichloromethane (DCM), and ethyl acetate (EA) extracts and were then tested for their cytotoxic activities on MCF7 and MCF7/HER2 cells using MTT assay. The most potential extracts were then examined for cytotoxicity in combination treatment with doxorubicin followed by cell cycle and apoptosis analysis with flow cytometry. All the extracts showed low and no cytotoxic activities against both MCF7 and MCF7/HER2 cells. The DCM extract exhibited a weak cytotoxic activity on both cancer cells with the IC_{50} values of 220 µg/ml. However, DCM and EA extracts gave synergistic cytotoxic effects with Dox on both cells with strong synergism characteristics. Both extracts induced cell cycle accumulation on the S-phase for DCM and the G1 phase for EA. Both extracts also caused apoptosis with Dox but in different ways in modulating apoptosis. In conclusion, DCM and EA extract of *V. amygdalina* provide potential synergistic anticancer properties with Dox for luminal breast cancer through cell cycle modulation and apoptosis induction.

INTRODUCTION

Breast cancers with ER expression and HER2 amplification are still challenging to achieve the best therapy for patients who have been experiencing resistance to drugs or other medications that reduce their efficacy [1]. Namely tamoxifen, the specifically targeted treatment for Estrogen Receptor [2], the usage routinely decreases the sensitivity of cancer cells and causes cancer cells to become resistant [3]. This phenomenon usually happens when the cancer cells change the growth signaling to another pathway rather than ER [4]. The HER2-targeted drugs also show a similar effect that the cancer cells do not respond to the drugs properly due to the use of a different signaling pathway to induce cell division [1,5]. Therefore, the use of generally targeted chemotherapy such as doxorubicin is still the drug of choice.

Doxorubicin is the most common treatment of cancer including luminal breast cancers [6]. However, the use of doxorubicin is limited due to raising the risk for cardiac and heart disease [7]. Doxorubicin is highly disposed of in cardiac cells and induces ROS generation leading to increasing cellular and tissue disruption [8]. This condition should be considered and given special attention when the use of doxorubicin in cancer treatment [9]. The use of ROS-reducing agents will give significant benefits

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to the co-treatment of doxorubicin in combating cancers [10]. Several natural products have been reported and give promising benefits for this purpose. Hesperidin increases the cytotoxic activity of doxorubicin in several cancer cell lines, such as 4T1 [11], MCF-7, and T47D [12]. The same results have also been achieved by galangal extract [13,14] or rice bran extract [15]. The documentation is still limited and needs to expand more rigorously. In this regard, we challenge the Africa leaf herb (*Vernonia amygdalina*) to be the co-treatment of doxorubicin on MCF-7 and MCF-7/HER2. MCF-7 is known as an estrogen receptor (ER) positive breast cancer cell line [16], whereas MCF-7/HER2 is HER2 transfected MCF-7 to make transiently high expressed HER2 [17].

Africa leaf (*V. amygdalina*) also known as bitter leaf, is a plant that contains compounds such as saponins, flavonoids such as luteolin, and steroid glycosides (cardiac glycosides), including vernodalin, vernon amygdalin, vernonioside B1, and vernoniol B1[18,19]. Experimental animal studies have demonstrated various activities of these compounds, including antioxidant, antimutagenic, anticancer, antidiabetic, antibacterial, and analgesic activities [20,21]. The 80% ethanol extract of *V. amygdalina* showed a cytotoxic effect at a dose of 100 µg/ml on MCF-7 cells and 50 µg/ml on MDA-MB-231 cells, besides the extract resulted in cell cycle arrest in the G1/S phase in MCF cells-7 and triggers apoptosis in both cells, especially at a concentration of 100 µg/ml [22]. The findings indicate a modest potential; hence, it is imperative to undertake further investigation through fractionation to identify specific groups of compounds demonstrating optimal potential, notably flavonoids. These compounds, in general, may also be explored for research purposes as co-chemotherapy agents. Flavonoids present in the Africa leaf exhibit anti-inflammatory [23], antiviral [24], and antioxidant [25]. The cardiac glycosides of *V. amygdalina* are potential compounds to protect against cardiotoxicity by inhibiting the Na+/K+ pump [26], whereas the ethanolic extract of *V. amygdalin*a which is rich in flavonoid luteolin content and cardiac glycosides show significant inhibitory effect of doxorubicin induced-cardiotoxicity [27]. These facts open the opportunity to use *V. amygdalina* as a combination chemotherapy with doxorubicin. The active compound of Africa leaf, vernodalinol, has been studied on MCF-7 cell lines, but only in cytotoxic assay with IC_{50} value of 70–75 µg/ml [28]. This study investigates various fractions of Africa leaves and their physiological impacts. Moreover, an investigation into the potential synergism between these extracts and doxorubicin is conducted. The aim of this study is to provide a novel alternative application of *V. amygdalina* extract by enhancing the effectiveness of doxorubicin through this combination.

METHOD

Materials and extraction

The extract preparation was conducted through reflux utilizing n-hexane as a solvent, followed by a subsequent step employing methanol. The methanol extract obtained was then fractionated using dichloromethane (DCM), ethyl acetate (EA), and n-butanol (BuOH) as solvents, employing liquid–liquid extraction methods to obtain DCM extract, EA extract, and BuOH extract [21].

Cell culture

The luminal breast cancer cell models, MCF-7 and MCF-7/HER2, were obtained from the cell line collection of Cancer Chemoprevention Research Center, Universitas Gadjah Mada, Indonesia. The cells were maintained using high-glucose DMEM (Gibco) with supplementary of 10% fetal bovine serum (Gibco), 1.5% penicillin–streptomycin (Gibco), and incubated in a CO_2 incubator 5% at 37°C.

Cytotoxic MTT assay

Each extract of Africa leaves (*V. amygdalina*) cytotoxicity was conducted on MCF-7 and MCF-7/HER2 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were grown at a density of 4×10^3 cells per well of a 96-well plate and incubated for 24 hours. Subsequently, the cells underwent treatment with varying concentrations of extracts (ranging from 1 to 500 µg/ml) and were incubated for 24 hours. Following the incubation period, the MTT (Sigma) reagent was introduced, and the absorbance was measured at 595 nm using a multi-plate reader (BioRad). Employing Hill's equation, the absorbance data were transformed into percentage cell viability, facilitating the determination of the IC_{50} value. Besides, the cytotoxic combination assay was determined using isobologram analysis to acquire the combination index (CI) value. CI values determined using CompuSyn software based on the Chou-Talalay system show the effects of drug combinations.

Cell cycle and apoptosis induction by flow cytometry assay

MCF-7 and MCF-7/HER2 cells were grown in a 6-well approximately 5×10^5 cells/well plate and incubated for 24 hours. The cells were treated with the extract and doxorubicin, either individually or in combination for 24 hours. Cell harvesting was performed using trypsin EDTA, followed by washing with phosphate-buffered saline (PBS) and centrifugation at 500 rpm for 5 minutes. For apoptosis induction, the cells were incubated with annexin-V-FITC and propidium iodide (PI) (BD Pharmingen), and then the analysis was conducted using the BD Accuri C6 Flow cytometer. To assess cell cycle distribution, the cells were fixed with cold 70% ethanol for 30 minutes, washed with PBS, and centrifuged at 500 rpm for 5 minutes. Subsequently, the cells were resuspended in PBS containing 40 µg/ml PI (Sigma), 20 µg/ml RNAse (Roche), and 0.1% TritonX-114 (Sigma). The resuspended cells were then subjected to analysis using the BD Accuri C6 flow cytometer.

RESULT

Cytotoxic activity of several extracts of Daun Afrika

This study intended to the challenge of cytotoxic combination (co-treatment) of Africa leaf extracts and doxorubicin on MCF-7 and MCF-7/HER2. First, we examined the cytotoxic activities of Hex, BuOH, DCM, MeOH, and EA extracts on both cells using MTT Assay. The results show that all of the extracts have low cytotoxicity with IC_{50} values of more than 200 µg/ml. Among the extracts, DCM extract shows the most cytotoxic potential on MCF-7 and MCF-7/HER2 with

an IC_{50} value of 220 µg/ml (Fig. 1A) and 220 µg/ml (Fig. 1B), respectively. These IC_{50} values indicate that this extract exhibits weak cytotoxic activity toward both test cells. BuOH has a low IC_{50} value, but only in MCF-7 cells. Therefore, no further tests were carried out. Whereas, on a vero normal cell, all of the extracts do not show a cytotoxicity effect (Fig. 1C). All these cytotoxic results show in Figure 1D.

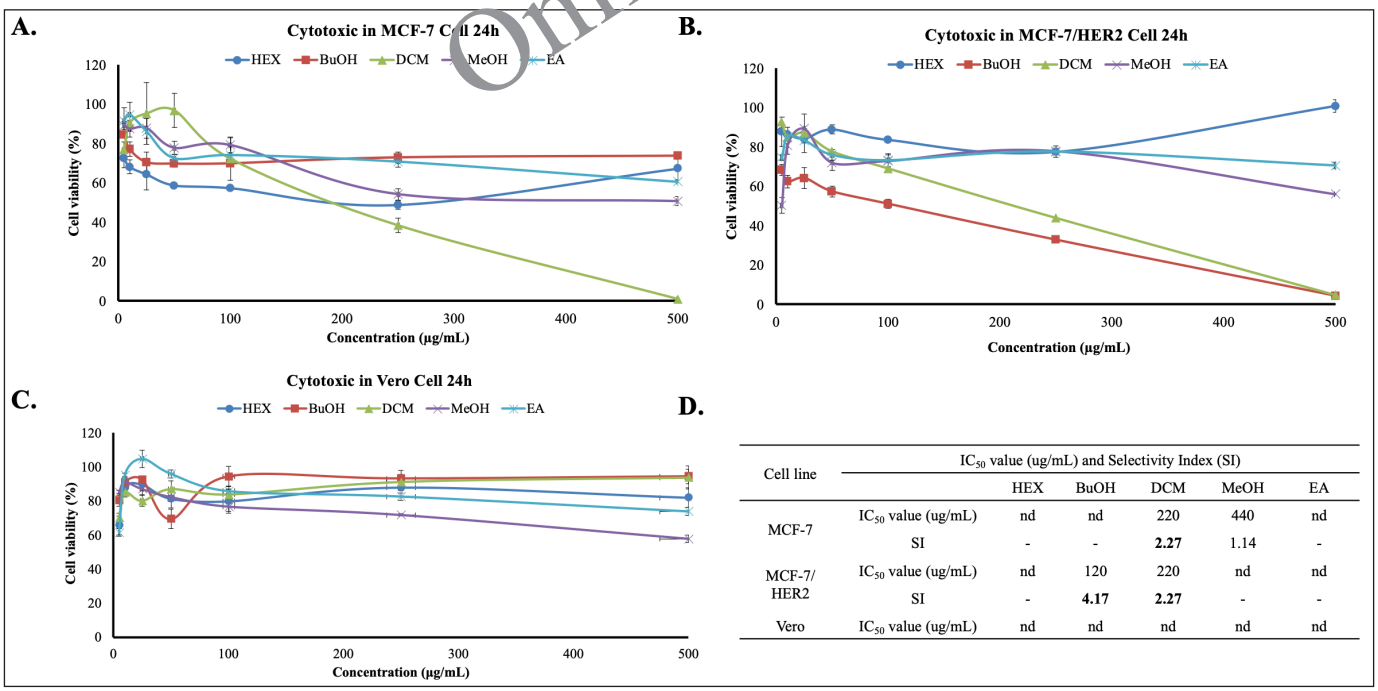
Cytotoxic combination of DCM and EA

In a single cytotoxicity test, DCM has strong potential despite its weak cytotoxicity. Then, we examined the cytotoxic combination with doxorubicin to reveal the synergy against breast cancer cells. The result shows that DCM in combination with doxorubicin has good synergistic properties on MCF-7 (Fig. 2A) and MCF7/HER2 (Fig. 2B) cells in inhibiting cell growth with a CI value of less than 0.3. In addition, we examined EA in MCF-7 cells, even though EA is weakly cytotoxic. On the contrary, EA has an antagonistic effect when combined with doxorubicin against MCF-7/HER2 (data not shown), but EA provides synergistic effect on MCF-7 (Fig. 2C). Therefore, further analysis was carried out to determine the cell cycle profile to see the physiological changes related to the lowering cell viability mediated by DCM alone or in combination with doxorubicin on MCF-7 and MCF-7/HER2.

Cell cycle modulation and apoptosis induction of MCF-7 by DCM and EA in combination with doxorubicin

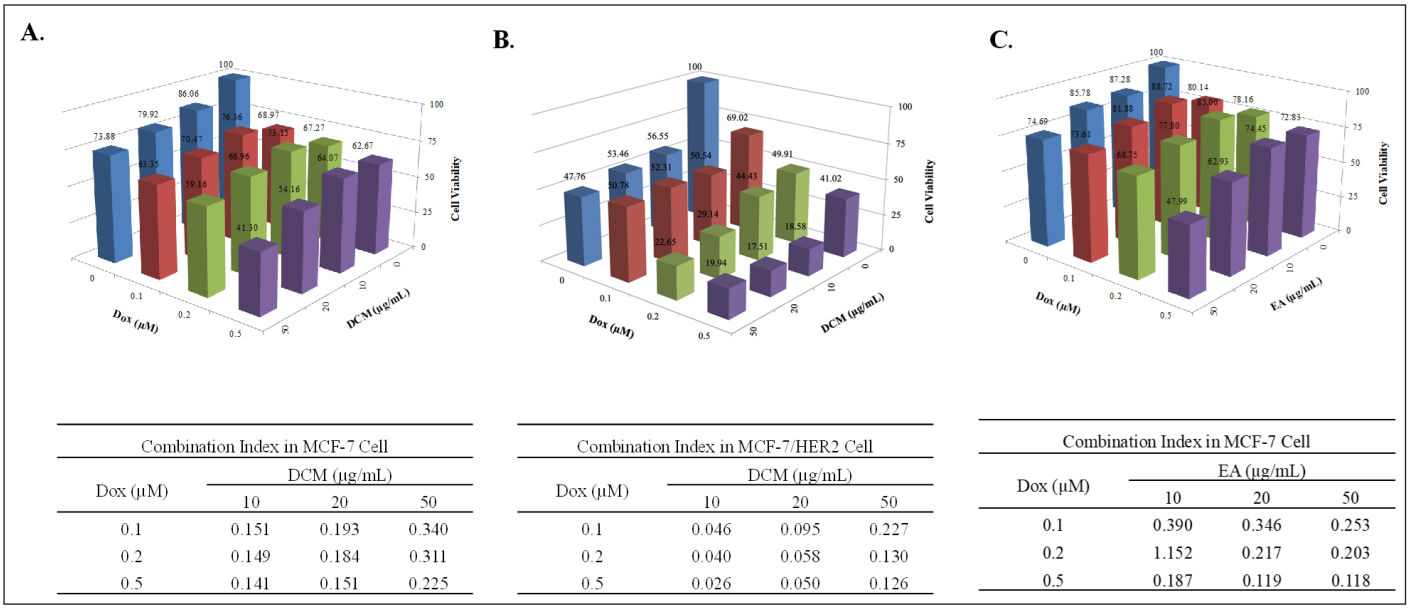
The synergistic effect on inhibiting cell viability by the combination of DCM, EA, and doxorubicin may be caused by the modulation of cellular physiological processes. To investigate the effect of this alone or combined treatment with doxorubicin on specific cellular physiological processes, we conducted additional investigations into MCF-7 cell cycle progression and apoptosis induction. The total distribution of cells in each phase was measured by flow cytometry with propidium iodide (PI) staining after treatment of cells with half dose (110 µg/ml DCM, 250 µg/ml DCM) and the combination with 250 nM Doxorubicin for 24 hours (Fig. 3A). The results show that a single treatment of DCM accumulated in the S phase, while a single doxorubicin accumulated in the Sub G1 phase. Interestingly, doxorubicin in a single treatment has low accumulation in the G2/M phase, yet in combination with DCM has high accumulation in the G2/M phase (Fig. 3B).

Furthermore, we investigated the apoptosis induction of DCM in combination with doxorubicin to reveal another cellular physiological process (Fig. 4A). The result of EA shows that EA accumulated in the G1 phase, but when its combination with Doxorubicin accumulated in Sub G1 phase, following doxorubicin, it was different from DCM. Hereafter, to find out whether cell death occurred through apoptosis, an apoptosis test was carried out by staining Annexin V-FITC and PI on MCF-7 cells and analyzing by flow cytometry. The single doxorubicin treatment has high levels of apoptosis, both in early and late apoptosis. The high levels of living cells in EA treatment indicate that EA causes cell cycle arrest because the G1 phase is high, however when it is combined with doxorubicin causes early apoptosis (Fig. 3D). The high levels of living cells in DCM treatment also indicates that DCM causes cell cycle arrest in



Cell line	IC_{50} value (ug/mL) and Selectivity Index (SI)	HEX	BuOH	DCM	MeOH	EA
MCF-7	IC_{50} value (ug/mL)	nd	nd	220	440	nd
	SI	-	-	**2.27**	1.14	-
MCF-7/ HER2	IC_{50} value (ug/mL)	nd	120	220	nd	nd
	SI	-	**4.17**	**2.27**	-	-
Vero	IC_{50} value (ug/mL)	nd	nd	nd	nd	nd

Figure 1. Cytotoxic effects of Africa Leaves (*V. amygdalina*) extracts on MCF-7 and MCF-7/HER2 Cells for 24 hours. (A) MCF-7, (B) MCF-7/HER2, (C) Vero cells were cultured in 96-well plates for 24 hours, then exposed to varying concentrations (5–500 µg/ml) of the HEX extract, BuOH extract, DCM, MeOH, and EA extracts. The cell viability was conducted through the MTT assay in triplicate (n = 3). (D) The IC_{50} value and SI (Selectivity Index) of each extract. The SI is calculated by comparing the IC_{50} of the extract against Vero cells and MCF-7 nor MCF-7/HER2 cancer cells.



Combination Index in MCF-7 Cell			
Dox (µM)	DCM (µg/mL)		
	10	20	50
0.1	0.151	0.193	0.340
0.2	0.149	0.184	0.311
0.5	0.141	0.151	0.225

Combination Index in MCF-7/HER2 Cell			
Dox (µM)	DCM (µg/mL)		
	10	20	50
0.1	0.046	0.095	0.227
0.2	0.040	0.058	0.130
0.5	0.026	0.050	0.126

Combination Index in MCF-7 Cell			
Dox (µM)	EA (µg/mL)		
	10	20	50
0.1	0.390	0.346	0.253
0.2	1.152	0.217	0.203
0.5	0.187	0.119	0.118

Figure 2. The combined effect of the DCM extract (A&B) or EA extract (C) from Africa Leaves (*V. amygdalina*) and doxorubicin (Dox) treatment on MCF-7 (A&C) and MCF-7/HER2 (B) cells. The cells were cultured in 96-well plates for 24 hours and exposed to ⅛, ¼, and ½ IC_{50} concentrations of DCM extract and Dox for an additional 24 hours then measured the cell viability (Upper panels). The synergistic effect when the DCM extract is combined with Dox, as indicated by a CI value of less than 0.3 (Lower panels).

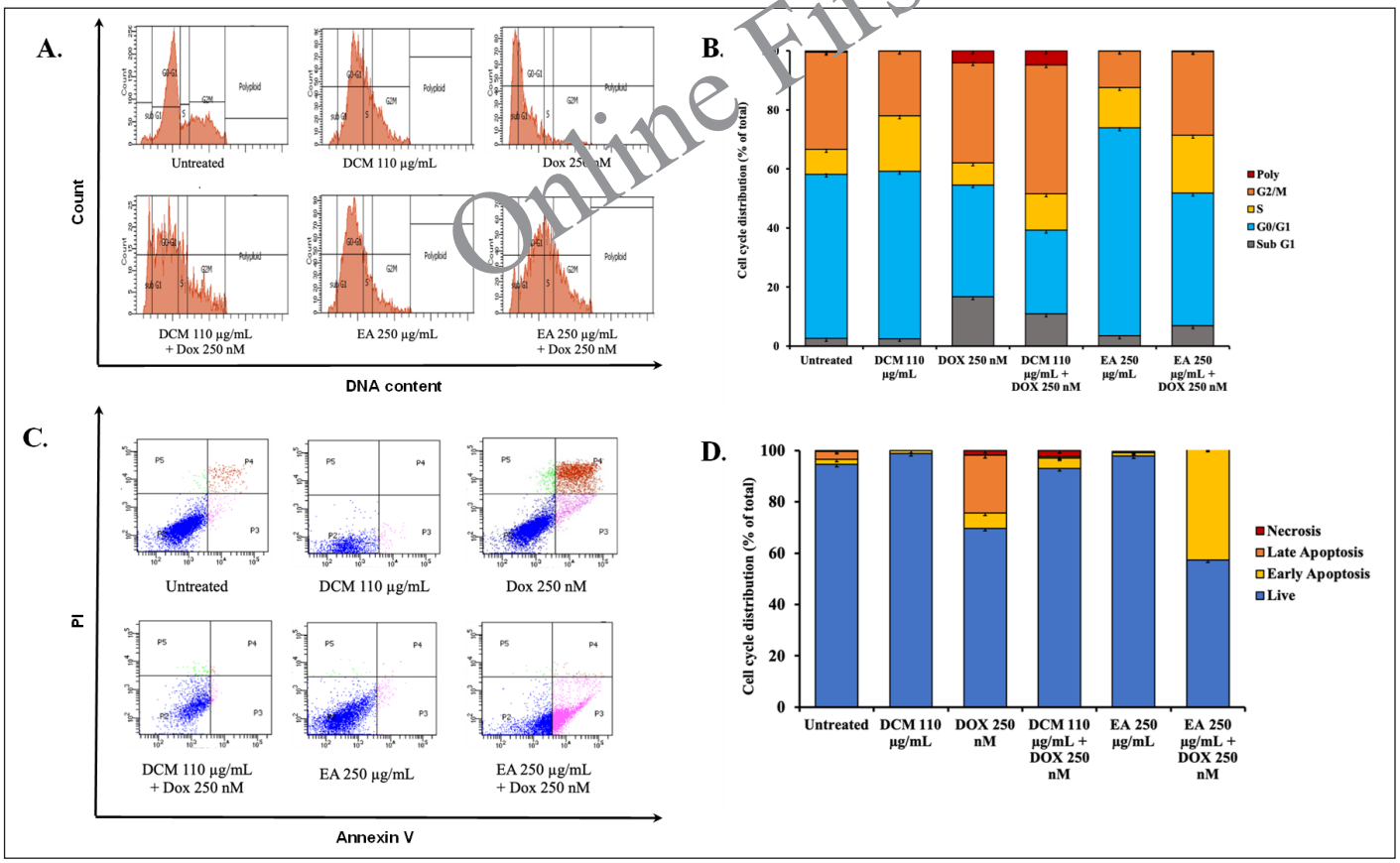


Figure 3. The cell cycle distribution and apoptotic profile of MCF-7 cells over a 24 hour treatment period. (A) The cell cycle profile with half the IC_{50} extract (DCM and EA), doxorubicin, and a combination of both compounds in MCF-7 cells for 24 hours incubation. PI solution was added to the cells, and the samples were analyzed using flow cytometry. (B) The distribution of MCF-7 cells across different phases of the cell cycle. (C) The effect of ½ IC_{50} extracts (DCM and EA), doxorubicin, and a combination of these compounds over 24 hours in MCF-7 cells. The cells were treated for 24 hours, stained with PI reagent, and each sample was analyzed using a flow cytometer. (D) The results of the analysis, illustrate the apoptosis induction for each treatment in MCF-7 cells.

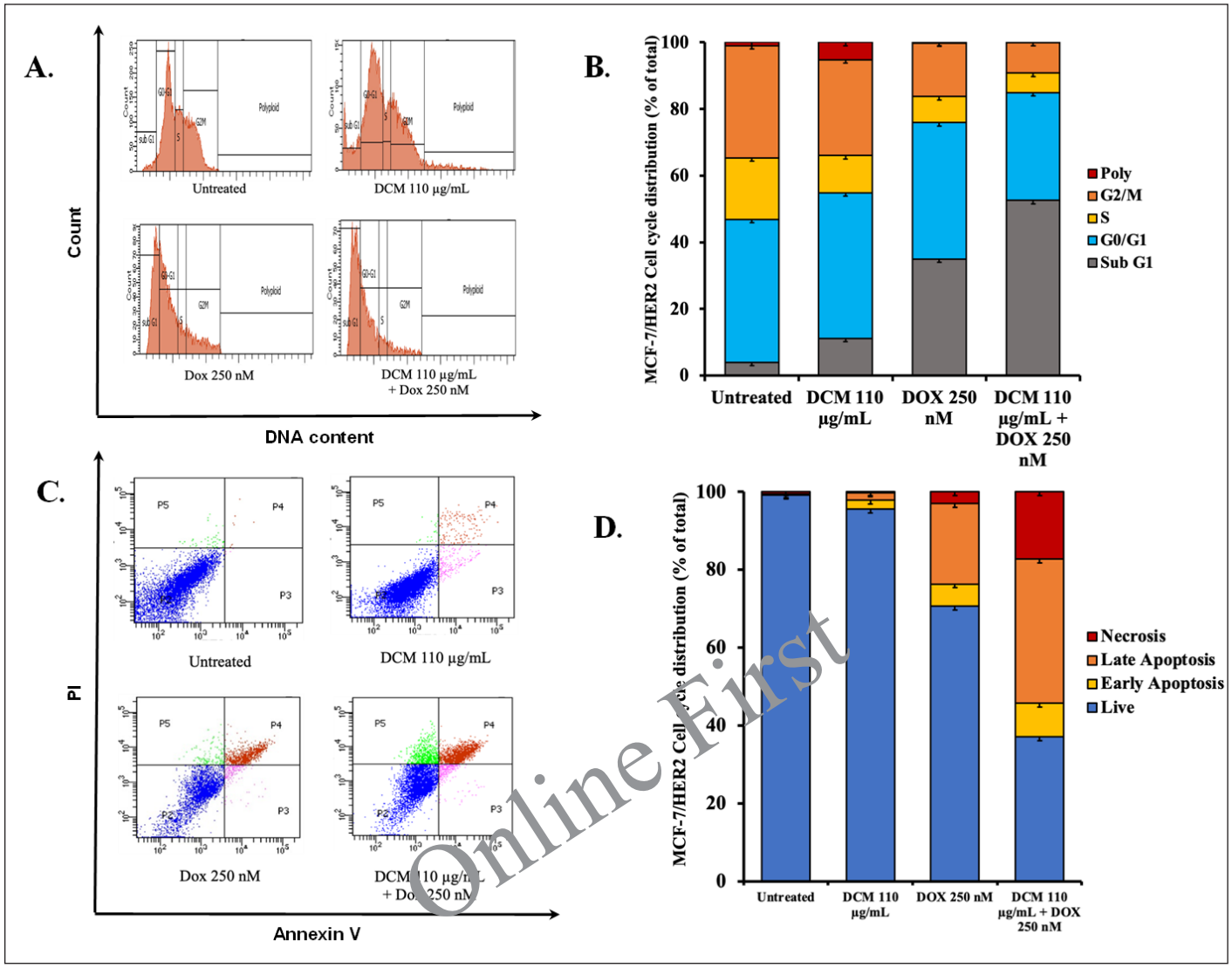


Figure 4. The cell cycle distribution and apoptotic profile of MCF-7/HER2 cells over a 24 hour treatment period. (A) The cell cycle profile is presented after a 24 hours incubation with DCM and Dox in MCF-7/HER2 cells, with the analysis conducted using PI staining and flow cytometry. (B) The distribution of MCF-7/HER2 cells across different phases of the cell cycle. (C) The effect of ½ IC_{50} extract (DCM only), doxorubicin, and a combination of these compounds over 24 hours in MCF-7 cells, stained with PI reagent, and subjected to flow cytometry analysis. (D) The results of the analysis, illustrate the apoptosis induction for each treatment in MCF-7/HER2 cells.

G2/M phase. Therefore, the inhibiting cell of MCF-7 viability is not due to cell death but tends to undergo cell cycle arrest.

Cell cycle modulation and apoptosis induction of MCF-7/HER2 by DCM in combination with doxorubicin

We also investigated the physiological processes of DCM treatment whether single or combination with doxorubicin on MCF-7/HER2 cells. The cell cycle of untreated MCF-7 cells shows a normal cell cycle (Fig. 4A), whereas in single DCM treatment shows a slight change in increasing Sub G1 phase (11%). Meanwhile, there was a change in doxorubicin treatment, increasing the level in the Sub G1 phase (35%). Even more surprising, DCM in combination with doxorubicin shows a drastic increase in the Sub G1 phase (52.6%) (Fig. 4B). Therefore, an apoptosis flow cytometry approach using Annexin-V is needed. In this regard, we can see that qualitatively between control and treated cells show a different distribution of cells in each quadrant (Fig. 4C). After counting these distributions, all of the untreated cells still live. DCM treatment is less apoptotic than the single doxorubicin treatment. Interestingly, when combined, the incidence of apoptosis increased significantly by 23% and additional necrosis by 8 % (Fig. 4D). These phenomena indicate a concordance with the increase in the Sub G1 phase of cell cycle assay and apoptosis in Annexin-V assay.

DISCUSSION

Vernonia amygdalina (Africa leaves) are rich in flavonoid luteolin and cardiac glycosides which is a potential source of antioxidant materials and cardiac toxicity protection. This pharmacological characteristic is important as a natural

resource to be used as a complementary medicine with some potentially toxic drugs to the cardiovascular system. Doxorubicin is one of the cardiotoxic drugs that should be considered for its application. Since doxorubicin is still the first line in chemotherapy, the use of doxorubicin should be accompanied by some protective agents to attenuate the toxicity to normal cells, including the disruption of cardiac cells. *Vernonia amygdalina* will give the opportunity to overcome this problem from the perspective of co-chemotherapeutics potential in luminal breast cancers.

Fortunately, all the fractionated extracts of *V. amygdalina* had no cytotoxic effect on normal cell lines. In this regard, we use a vero cell line that represents a normal kidney cell. In contrast, the DCM extract gave low cytotoxic activity against cancer cells, MCF-7 and MCF-7/HER2 but not the other extracts. This result indicates that *V. amygdalina* has a weak potential as an anticancer agent, especially for luminal breast cancer with ER and HER2 expression. This phenomenon is also the characteristic of herbal extract having a low cytotoxic effect against cancer cells which may be due to the low content of active compounds. Since DCM or EA extracts are relatively semi-polar solvents, these extracts usually contain rich flavonoids and glycoside compounds. We confirmed this fact in our previous study [26]. Luteolin and glycoside forms also do not include the strong cytotoxic agents but they have high antioxidant properties as radical scavengers. Therefore, these extracts could potentially be combined with doxorubicin as a co-chemotherapeutic agent.

The cytotoxicity of the combination treatment is the basic understanding of the potential of co-chemotherapeutics application. In this perspective, DCM extract performed a synergistic effect with doxorubicin on MCF-7 and MCF-7/HER2 cells, whereas EA extract also exhibited a synergistic effect with doxorubicin on MCF-7 cells. These results indicate that both extracts have the potential to be developed as co-chemotherapeutic agents with doxorubicin that need to be explored for further physiological impact. Cell cycle progression and apoptosis evidence will be the main effect due to the cytotoxic activities of the extracts which are closely related to the lowering of cell viability after combination treatments. Cell cycle progression correlates with cell division activity that can be stopped by the treatment, meanwhile, apoptosis can reduce cell viability by losing the living cells. Both physiological processes may go in sequential ways, but they can proceed irrespective of ways that are interesting to be explored.

We found that both cells, MCF-7 and MCF-7/HER2 give different responses regarding cell cycle progression and apoptosis evidence to DCM extract. The MCF-7 cells tend to have an irrespective effect between cell cycle and apoptosis in combination treatment and seem to delay the apoptosis by DCM through cell cycle arrest in the G2/M phase. In contrast, the MCF-7/HER2 shows more sensitivity to undergo apoptosis in combination treatment, meaning that DCM probably has a role in enhancing the apoptosis process by doxorubicin. Nevertheless, the necrotic cells of MCF7/HER2 due to combination treatment could be the effect of accelerating the cell apoptosis evidence in early time and proceeding into necrosis. Both cells are only different in HER2 expression. Therefore, we could speculate that HER2 signaling may play a role in this phenomenon, and inhibition of HER2 signaling will be inhibited by compounds in DCM. This fact will be interesting for further investigation.

Nevertheless, all these limited findings are interesting to give insight into the potential usage of flavonoid and glycoside-rich extract of *V. amygdalina* in combination with doxorubicin. The future challenges are to explore in more detail the molecular mechanism underlying the disrupting proliferative signaling by flavonoid and its glycoside forms related to the ER and HER2 receptors. The ER and HER2 receptors are common inducers in cell division through MAPK pathways, whereas flavonoids are known to be able to inhibit some kinases. These phenomena could be the coincidence that is important to be elucidated, *in vitro* and *in vivo* approaches.

CONCLUSION

In conclusion, DCM synergistically lowers cell viability due to doxorubicin treatment on MCF-7 and MCF-7/HER2. In MCF-7 DCM tends to stop the cell cycle in the G2/M phase with doxorubicin, but DCM enhances the apoptosis caused by doxorubicin in MCF-7/HER2.

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AUTHOR CONTRIBUTIONS

NPH executing most of the experiment and preparing the manuscript; NN conducting part of the in vitro cytotoxic experiment; DRR supporting flowcytometry analysis; MH aided in the data analysis and interpretation; DS was responsible for the extract and supporting data acquisition; PA designed the work and supporting data acquisition; EM designed and supervised the work, analyzed the data, developed the writing flow, and revised the manuscript. All authors contributed to the critical revision of the manuscript.

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CONFLICTS OF INTEREST

The authors declared that they have no conflict of interest.

ETHICAL APPROVALS

The experimental protocol for this study was approved by the Ethics Committee of UGM, Indonesia (No. KE/FK/1004/EC/2023).

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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REFERENCES

1. Novitasari D, Jenie RI, Kato JY, Meiyanto E. Chemoprevention curcumin analog 1.1 promotes metaphase arrest and enhances intracellular reactive oxygen species levels on TNBC MDA-MB-231 and HER2-positive HCC1954 cells. Res Pharm Sci. 2023 Jul 1;18(4):358–70.
2. Hermawan A, Putri H. Identification of potential gene associated with berberine in overcoming tamoxifen resistance by functional network analysis. J Appl Pharm Sci. 2020 Jun 30;10(7):009-18.
3. Zhang Z, Park JW, Ahn IS, Diamante G, Sivakumar N, Arneson D, *et al*. Estrogen receptor alpha in the brain mediates tamoxifen-induced changes in physiology in mice. Elife. 2021 Mar 1;10:e63333.
4. Hermawan A, Ikawati M, Khumaira A, Putri H, Jenie RI, Angraini SM, *et al*. Bioinformatics and *in vitro* studies reveal the importance of p53, PPARG and notch signaling pathway in inhibition of breast cancer stem cells by hesperetin. Adv Pharm Bull. 2021 Feb;11(2):351.
5. Handayani S, Susidarti RA, Utomo RY, Meiyanto E, Jenie RI. Synergistic cytotoxic and antimigratory effect of brazilein and doxorubicin on HER2-overexpressing cells. Asian Pac J Cancer Prev. 2022 Aug;23(8):2623.
6. Nurhayati IP, Khumaira A, Ilmawati GP, Meiyanto E, Hermawan A. Cytotoxic and antimetastatic activity of hesperetin and doxorubicin combination toward Her2 expressing breast cancer cells. Asian Pac J Cancer Prev. 2020 May;21(5):1259.
7. Wulandari F, Novitasari D, Kirihata M, Kato JY, Meiyanto E. New curcumin analog, CCA-1.1, synergistically improves the antiproliferative effect of doxorubicin against T47D breast cancer cells. Indones J Pharm. 2020;31:244–56.
8. Ikawati M, Jenie RI, Utomo RY, Amalina ND, Ilmawati GP, Kawaichi M, *et al*. Genistein enhances cytotoxic and antimigratory activities of doxorubicin on 4T1 breast cancer cells through cell cycle arrest and ROS generation. J Appl Pharm Sci. 2020 Oct 5;10(10):095–104.
9. Rawat PS, Jaiswal A, Khurana A, Bhatti JS, Navik U. Doxorubicin-induced cardiotoxicity: an update on the molecular mechanism and novel therapeutic strategies for effective management. Biomed Pharmacother. 2021 Jul 1;139:111708.
10. Salsabila IA, Nugraheni N, Ahlina FN, Haryanti S, Meiyanto E. Synergistic cotreatment potential of soursop (*Annona muricata* L.) leaves extract with Doxorubicin on 4T1 cells with antisenescence and anti-reactive-oxygen-species properties. Iran J Pharm Res. 2021;20(2):57.
11. Amalina ND, Salsabila IA, Zulfin UM, Jenie RI, Meiyanto E. *In vitro* synergistic effect of hesperidin and doxorubicin downregulates epithelial-mesenchymal transition in highly metastatic breast cancer cells. J Egypt Natl Cancer Inst. 2023 Dec;35(1):6.
12. Hermawan A, Putri H. Targets and molecular mechanisms of a citrus flavonoid, hesperidin, against luminal breast cancer cells: an integrative bioinformatics analysis. Asian Pac J Trop Biomed. 2019 Dec 1;9(12):531–8.
13. Ahlina FN, Nugraheni N, Salsabila IA, Haryanti S, Da'i M, Meiyanto E. Revealing the reversal effect of galangal (*Alpinia* galanga L.) extract against oxidative stress in metastatic breast cancer cells and normal fibroblast cells intended as a co-chemotherapeutic and anti-ageing agent. Asian Pac J Cancer Prev. 2020 Jan 1;21(1):107–17.
14. Alif I, Utomo RY, Ahlina FN, Nugraheni N, Hermansyah D, Putra A, *et al*. Immunopotentiation of galangal (*Alpinia* galanga L.) when combined with T-cells against metastatic triple-negative breast cancer, MDA-MB 231. J Appl Pharm Sci. 2021 Jul 18;11(11):053–61.
15. Zulfin UM, Rahman A, Hanifa M, Utomo RY, Haryanti S, Meiyanto E. Reactive oxygen species and senescence modulatory effects of rice bran extract on 4T1 and NIH-3T3 cells co-treatment with doxorubicin. Asian Pac J Trop Biomed. 2021 Apr 1;11(4):174–82.
16. Comşa Ş, Cimpean AM, Raica M. The story of MCF-7 breast cancer cell line: 40 years of experience in research. Anticancer Res. 2015 Jun 1;35(6):3147–54.
17. Zhao S, Ohara S, Kanno Y, Midorikawa Y, Nakayama M, Makimura M, *et al*. HER2 overexpression-mediated inflammatory signaling enhances mammosphere formation through up-regulation of aryl hydrocarbon receptor transcription. Cancer Lett. 2013 Mar 1;330(1):41–8.
18. Nowak J, Kiss AK, Wambebe C, Katuura E, Kuźma Ł. Phytochemical analysis of polyphenols in leaf extract from *Vernonia amygdalina* Delile plant growing in Uganda. Appl Sci. 2022 Jan 17;12(2):912.
19. Ondua M, Njoya EM, Abdalla MA, McGaw LJ. Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. J Ethnopharmacol. 2019 Apr 24;234:27–35.
20. Okello D, Chung Y, Kim H, Lee J, Rahmat E, Komakech R, *et al*. Antioxidant activity, polyphenolic content, and FT-NIR analysis of different *Aspilia africana* medicinal plant tissues. Evid Based Complement Altern Med. 2021 Sep 15;2021:e9917810.
21. Hasibuan PA, Harahap U, Sitorus P, Satria D. The anticancer activities of *Vernonia amygdalina* Delile. Leaves on 4T1 breast cancer cells through phosphoinositide 3-kinase (PI3K) pathway. Heliyon. 2020 Jul 1;6(7):e04449.
22. Wong FC, Woo CC, Hsu A, Tan BK. 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. 2013 Oct 24;8(10):e78021.
23. Owor RO, Bedane KG, Openda YI, Zühlke S, Derese S, Ong'amo G, *et al*. Synergistic anti-inflammatory activities of a new flavone and other flavonoids from *Tephrosia hildebrandtii* Vatke. Nat Prod Res. 2021 Nov 18;35(22):4486–93.
24. Kim CH, Kim JE, Song YJ. Antiviral activities of quercetin and isoquercitrin against human herpesviruses. Molecules. 2020 May 26;25(10):2379.
25. Sugiura Y, Katsuzaki H, Imai K, Amano H. The anti-allergic and anti-inflammatory effects of phlorotannins from the edible brown algae, *Ecklonia* sp. and *Eisenia* sp. Nat Prod Commun. 2021 Nov;16(12):1934578X211060924.
26. Harahap U, Dalimunthe A, Haro G, Syahputra RA, Utomo DH, Satria D. *In-silico* analysis of cardiac glycosides from *vernonia amygdalina* delile. leaves as cardiotonic through inhibition of NA+/K+ ATPase ion transport. Rasayan J Chem. 2021 Jan 1;14(1):161–5.
27. Syahputra RA, Harahap U, Harahap Y, Gani AP, Dalimunthe A, Ahmed A, *et al*. *Vernonia amygdalina* ethanol extract protects against doxorubicin-induced cardiotoxicity via TGFβ, cytochrome c, and apoptosis. Molecules. 2023 May 24;28(11):4305.
28. Luo X, Jiang Y, Fronczek FR, Lin C, Izevbigie EB, Lee KS. Isolation and structure determination of a sesquiterpene lactone (vernodalinol) from *Vernonia amygdalina* extracts. Pharm Biol. 2011 May 1;49(5):464–70.

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