Majalah Obat Tradisional (Trad. Med. J.), January-April 2026
Vol. 31(1), p 72-8
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 26-12-2024
Revised : 07-06-2025
Accepted : 06-10-2025

Anticancer Synergy of Myricetin and Doxorubicin in Breast Cancer: Network Pharmacology and In Vitro Evaluation

Roihatul Mutiah[1], Burhan Ma'arif[1], Indah Rahmatul Inayah[1], Avin Ainur Fitrianingsih[2*]
[1]Department of Pharmacy, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim Malang, East Java, Indonesia
[2]Department of Medicine, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim Malang, East Java, Indonesia

ABSTRACT

Breast cancer is a major public health problem worldwide, leading to an urgent need for the development of new strategies to improve treatment efficacy and manage drug resistance. Doxorubicin is among the most commonly used chemotherapy drugs and has the disadvantage of being easy resistance and toxicity, which causes limited efficiency. A potentially effective strategy is combining doxorubicin with natural compounds possessing anticancer activity and the ability to modulate drug resistance pathways. Therefore, this study aimed to explore the potential of combining myricetin and doxorubicin as an effective and safe anticancer treatment. A network pharmacology method was used to examine how the two compounds interact at the molecular level, including the target genes, signaling pathways, and possible synergistic effect. Additionally, laboratory (in vitro) tests were performed using MTT assay to evaluate the toxicity of the compounds to both T47D breast cancer and normal Vero cells. Several key parameters, including IC50 values of each compound, as well as the combination index and selectivity index (SI), were analyzed to evaluate the safety against normal cells. The results showed that the combination of myricetin and doxorubicin targeted 19 interconnected genes triggering apoptosis often associated with cancer treatment. Laboratory tests (in vitro) found that myricetin and doxorubicin had moderate anticancer or cytotoxic activity, with IC50 values of 31.936 µg/mL and 27.39 µg/mL, respectively. The combination at concentrations of 7.984 µg/mL myricetin + 3.424 µg/mL doxorubicin, 15.986 µg/mL myricetin + 3.424 µg/mL doxorubicin, and 31.936 µg/mL myricetin + 3.424 µg/mL doxorubicin had a powerful synergistic effect as well as high viability in Vero cells with a value of 168.756 µg/mL. Based on the determined combination concentrations and IC50 values of the compounds, myricetin, and doxorubicin showed potential synergistic activity against breast cancer cells. Breast cancer remains a significant global health challenge, necessitating the development of novel strategies to enhance treatment efficacy and combat drug resistance. Doxorubicin, a common chemotherapeutic drug, has limitations owing to its resistance and toxicity. This study investigated the potential of combining myricetin, a natural compound, with doxorubicin to address these issues. Employing a network pharmacology approach, we first explored the molecular interactions between myricetin and doxorubicin and identified target genes, signaling pathways, and potential synergistic effects. Concurrently, in vitro experiments using the MTT assay assessed the toxicity of the compounds in T47D breast cancer cells and normal Vero cells. The key parameters analyzed included the IC50 values, combination index, and selectivity index to evaluate safety. Our findings revealed that the combination of myricetin and doxorubicin targeted 19 interconnected genes that are frequently associated with apoptosis during cancer treatment. In vitro tests showed that both myricetin and doxorubicin exhibited moderate cytotoxic activity, with IC50 values of 31.936 µg/mL and 27.39 µg/mL, respectively. Specific combinations (e.g., 7.984 µg/mL myricetin + 3.424 µg/mL doxorubicin) demonstrated a powerful synergistic effect against breast cancer cells, while maintaining high viability in normal Vero cells (168.756 µg/mL). These results suggested that the combination of myricetin and doxorubicin offers a promising synergistic approach for breast cancer treatment.

Keywords: Doxorubicin; Myricetin; Network Pharmacology; T47D Cells; Vero Cells

INTRODUCTION

Cancer is a disease that is difficult to cure and can even cause death, leading to fear among many people (Yudistira, 2017). There are 18.1 million new cases with a death rate of 9.6 million based on Global Burden Cancer (Globocan) data in 2018. Additionally, Indonesia ranks eighth in Southeast Asia regarding the number of cancer cases (Gusungi et al., 2020). Breast cancer can develop because of damage to DNA and genetic changes. This damage occurs due to exposure to estrogen, which triggers gene mutations, and few

***Corresponding author : Avin Ainur Fitrianingsih**
Email : avinainur@kedokteran.uin-malang.ac.id

cases of DNA defects are genetically inherited (Alkabban et al., 2024).

Treatment of cancer includes surgery, radiotherapy, and chemotherapy. However, the treatment efforts carried out against various cancer types have not succeeded effectively in curing the disease. A method to address this phenomenon is by applying combination chemotherapy, which merges phytochemical compounds from natural ingredients with chemotherapeutic agents to increase treatment effectiveness and reduce toxicity to normal tissues (Mutiah et al., 2018).

The phytochemical compounds include myricetin, which can be obtained from various sources, naturally through extraction from flavonoid-containing plants or pure compounds purchased from chemical or biotechnology companies. According to data from Sigma Aldrich, myricetin was obtained from the first isolation of Morella rubra bark (Song et al., 2021). Myricetin plays a role in disease management as an antioxidant and anti-inflammatory, contributing to cancer prevention through the regulation of angiogenesis, inflammation, cell cycle arrest, and apoptosis induction (Rahmani et al., 2023). This compound can be mixed with doxorubicin, which is considered the first-choice chemotherapeutic agent for breast cancer and has proven to have significant therapeutic potential. Doxorubicin is recognized among the most effective chemotherapy drugs approved by Food and Drug Administration (FDA) for treating various cancer types, but the usage has adverse side effect on patients.

This study aimed use to Network Pharmacology method to explore the treatment effect of myricetin and doxorubicin combination, followed by validation through in vitro anticancer activity and safety tests on T47D and Vero cells. The conducted exploration is expected to reduce toxicity to normal cells as well as determine the synergistic potential between myricetin compounds and doxorubicin drugs.

MATERIALS AND METHODS

Materials

The sample materials used were pure myricetin compounds purchased from Sigma Aldrich and doxorubicin drugs. Furthermore, those needed in the identification process were GeneCards (https://www.genecards.org). The materials used in MTT method included T47D cells (ATCC), Vero cells (ATCC), PBS (Gibco), Trypsin-EDTA (Gibco), RPMI media (Gibco), M199 media (Gibco), MK, DMSO 0.2% (Merck), SDS 10% (Merck), MTT 5 mg/mL (Sigma Aldrich), and ethanol 70% (Merck).

Methods

Network Pharmacology

Network pharmacology method was executed in stages starting with the collection of target genes from myricetin and doxorubicin compounds through GeneCards database (https://www.genecards.org/), followed by the collection of genes related to breast cancer disease using DisGenet database (https://www.disgenet.org/). The interconnection between target genes of the samples (myricetin and doxorubicin) and the disease collected was predicted through Venn diagram, then the results were visualized using Cytoscape. Protein-protein interaction (PPI) network STRING (https://STRING-db.org/) was developed from the visualization results to determine the relationship between various target genes in the samples playing a role in the biological pathway of breast cancer treatment. The signaling pathway was analyzed using KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway (https://www.genome.jp/kegg/pathway.html) and Gene Ontology (http://geneontology.org/).

Anticancer Activity and Safety Testing

A preparation comprising T47D (breast cancer) and Vero (normal) cells was heated in a water bath at 47°C for 2 to 3 minutes, then transferred to the respective media (RPMI for T47D, M199 for Vero) and incubated three to four hours at 35°C with 5% CO_2. Cancer cells were separated and incubated for another 3 to 4 hours. When cells reached 80% confluency, the media was discarded, washed twice with PBS, and trypsin-EDTA was added for separation. Cells were incubated for 3 minutes before stopping with RPMI (T47D) or M199 (Vero) media, followed by another incubation for 24 hours. Harvested cells were counted with a hemocytometer using a light microscope at 100x magnification. The counted T47D and Vero cells (123×10^4 cells/mL and 129×10^4 cells/mL, respectively) were positioned in wells containing RPMI (T47D) and M199 (Vero) media, with a total volume of 10 mL per well. Incubation was carried out for 24 hours at 37°C with 5% CO_2. Test solutions were prepared by dissolving myricetin and doxorubicin in DMSO to produce various concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL. These were applied to a plate, while the media in the wells were discarded, washed with PBS, and added to the test solutions with three replicates per concentration

before incubating for 24 hours. Approximately 100 µL of MTT solution was introduced into each well and incubated for four hours at 37°C. A 100 µL SDS stopper was added and incubated for 24 hours at room temperature under conditions without lighting. ELISA reading was carried out at a wavelength of 595 nm for analysis and cell viability was calculated using the following formula:

$$Cell\ viability = \frac{Treatment\ absorbance - Media\ control\ absorbance}{Cell\ control\ absorbance - Media\ control\ absorbance} X\ 100\%$$

The cell viability results obtained can be calculated as IC50 value for anticancer activity and CC50 value for normal cell toxicity using SPSS probit analysis. Cytotoxicity level is classified into four categories based on the American National Cancer Institute, namely highly toxic, moderate, weak, and non-toxic with IC50 values of ≤ 20 µg/mL, 21-200 µg/mL, 201-500 µg/mL, and ≥ 500 µg/mL, respectively (Nurdiani et al., 2023).

Combination Test

A concentration series of samples (myricetin) and chemotherapeutic agents (doxorubicin) for treatment (including cell control) was prepared. This consisted of four concentrations, including IC50, 1/2 IC50, 1/4 IC50, and 1/8 IC50. Quantitative combination index (CI) analysis was used to explain the effectiveness of the combination by applying the following equation:

$$Combination\ Index\ (\mathrm{CI}) = \frac{(D)1}{(Dx)1} + \frac{(D)2}{(Dx)2}$$

D)1 and (D)2 refer to two different concentrations of compounds with the same effect on the experimental results. Meanwhile, (DX) is a single concentration of one among both compounds that produces the same effect, and CI numbers obtained are interpreted in Table I.

Ethical Approval

This study received ethical approval from the Health Research Ethics Commission of the Faculty of Medicine, Maulana Malik Ibrahim State Islamic University Malang, with certificate number 39/02/EC/KEPK-FKIK/09/2023.

RESULTS

Potential Target Genes and PPI

GeneCards and DisGenet were used to find potential target gene compounds combining myricetin and doxorubicin for breast cancer treatment. GeneCards analysis showed 175 and 3,408 target genes from myricetin and doxorubicin, respectively. Based on DisGenet analysis, there were 300 target genes from 10 types of breast cancer with different codes. These included Breast Cancer (CUI: C0006142), Triple-Negative Breast Cancers (CUI: C3539878), Invasive Breast Cancer (CUI: C0853879), Breast Cancer Stage IV (CUI: C0278488), Estrogen Receptor-Positive Breast Cancer (CUI: C2938924), Estrogen Receptor-Negative Breast Cancer (CUI: C4733092), Ductal Breast Cancer (CUI: C1527349), Advanced Breast Cancer (CUI: C3495917), Carcinoma of Breast (CUI: C0678222), and Triple-Negative Breast Carcinoma (CUI: C4722518). The Venn diagram analysis comparing the target genes of the compounds and the disease showed potential overlap between 19 target genes (Figure 1A). Further analysis related to pharmacological network was carried out using STRING and Cytoscape tools. The results of the interaction network of 19 myricetin and doxorubicin proteins in breast cancer contained 19 nodes with 116 edges (Figure 1B). Meanwhile, the Cytoscape results showed 23 nodes and 59 edges, implying that myricetin, doxorubicin, and breast cancer had 59 ropes of relationships to 23 target genes (Figure 1C).

KEGG (Kyoto Encyclopedia of Genes and Genomes) Analysis and Gene Ontology (GO)

This study performed a signaling pathway analysis with KEGG on target genes obtained from overlapping Venn diagram between myricetin, doxorubicin, and breast cancer. Figure 2 shows that the two compounds affect several signaling pathways associated with apoptosis, such as Protein Processing in the endoplasmic reticulum (ER), Calcium, Intrinsic, Extrinsic, mitogen-activated protein kinase (MAPK), PI3K-AKT, NF-kB, and P53 signaling pathways.

Protein Processing in ER is responsible for ensuring the proper functioning of newly synthesized proteins. ER is the main protein folding and maturation location of eukaryotic cells. Proteins residing in ER and those sent to Golgi are synthesized in ribosomes attached to ER membrane (Adams et al., 2019).

Calcium pathway plays important roles in various biological processes, such as increasing reactive oxygen species (ROS) on a mechanical level in the mitochondria (Wu et al., 2021). Meanwhile, the extrinsic pathway, often targeted by PARP1 (Poly [ADP-Ribose] Polymerase 1), triggers apoptosis through the activation of specific receptors on the cell surface, such as those engaged in tumor necrosis factor (TNF) signaling (Jan & Chaudhry, 2015).

The intrinsic pathway is a type of signaling included in apoptosis primarily regulated by mitochondria. This responds to internal and

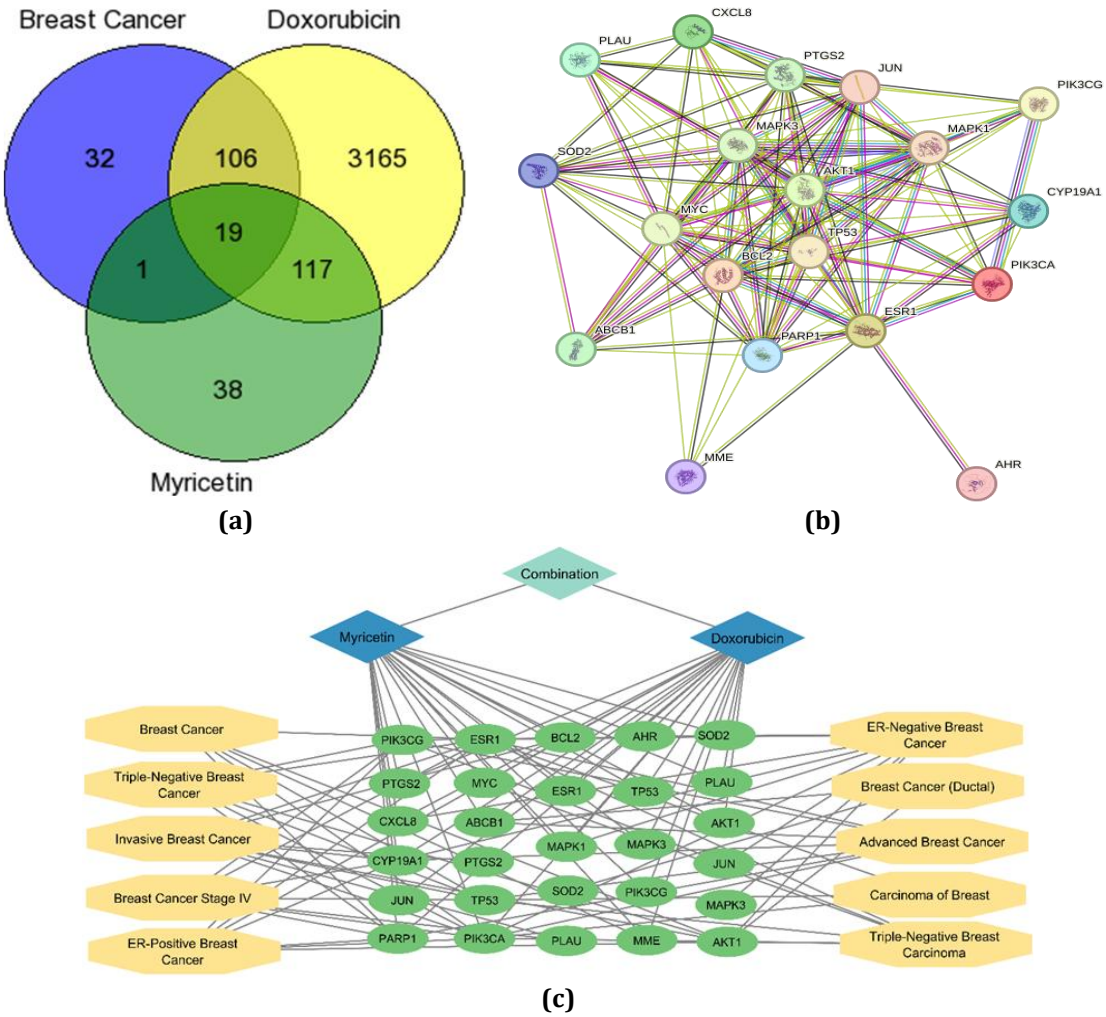

Figure 1. A) Venn diagram showing overlapping target genes, B) PPI Analysis (19 nodes, 116 rdges, PPI enrichment p-value: 1.28e-12), C) Network visualization of myricetin and doxorubicin combination (23 Nodes, 59 Edges) (Blue: compound name, Green: target protein, Cream: disease).

Table I. The Interpretation of CI

CI	Interpretation	CI	Interpretation
<0.1	Strong synergist	0.9-1.1	Additives
0.1-0.3	Powerful synergist	1.1-1.45	Weak antagonist
0.3-0.7	Synergist	1.45-3.3	Antagonist
0.7-0.9	Weak synergist	>3.3	Powerful antagonist

external forms of stress, such as oxidative stress, radiation, and exposure to cytotoxic drugs (Jan & Chaudhry, 2015). MAPK pathway plays a key role in regulating a wide range of cellular processes, including cell growth, specialization (differentiation), and the response of cells to stress (Park & Baek, 2022).

PI3K-AKT controls many important cellular processes, including metabolism, growth, survival, and cell division. This becomes activated in response to signals from outside the cell, triggering a chain reaction that starts with an enzyme called phosphatidylinositol 3-kinase (PI3K). The main targets in PI3K-AKT pathway include AKT proteins, which are a type of serine/threonine kinase existing in AKT1, AKT2, and AKT3 forms. Furthermore, the proteins help regulate how cells use glucose, stay alive, grow, and multiply (Nunnery & Mayer, 2020).

NF-kB pathway participates in the inflammatory response of the body and plays a role in determining the potential growth or death of a cell (Javed et al., 2022). Meanwhile, p53 signaling pathway is controlled by p53 protein,

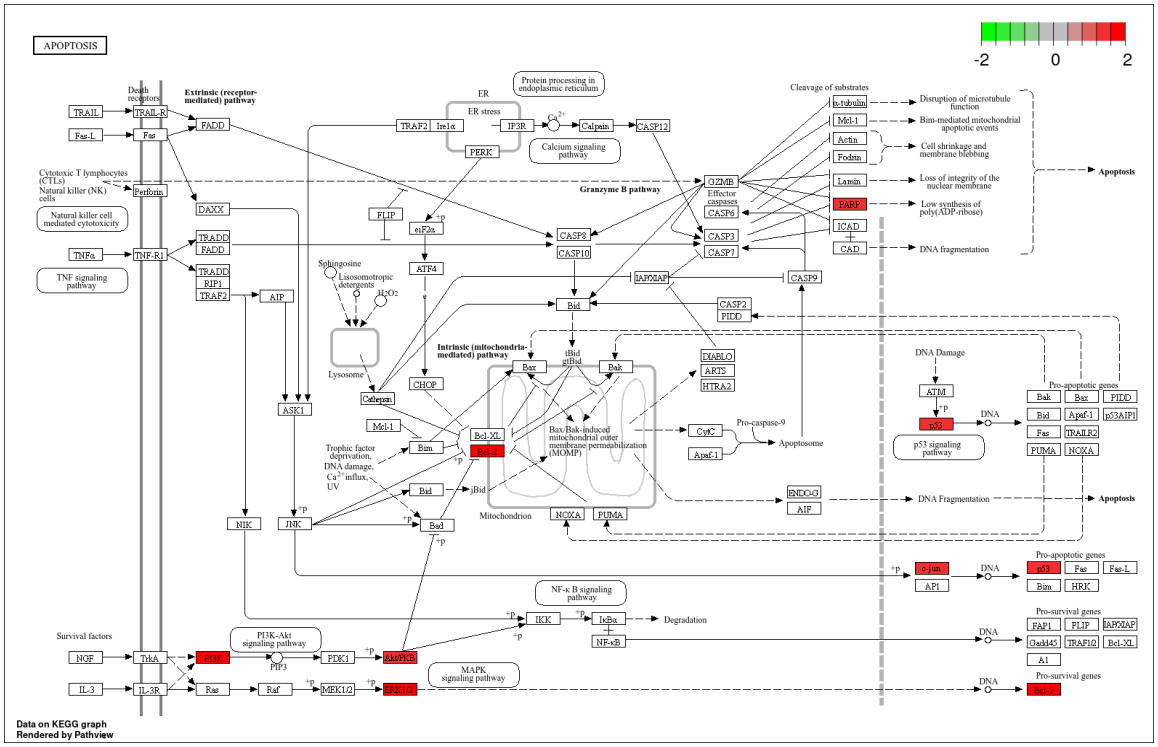

Figure 2. Apoptosis Pathway (hsa04210)

a transcription factor that activates the expression of several target genes. p53 plays an important role in the regulation of the cell cycle, apoptotic processes, and genome stability being widely regarded as the "guardian of the genome" (Wang et al., 2023).

Based on Figure 3, GO analysis showed that myricetin and doxorubicin compounds affected many biological processes, molecular functions, and cellular components. The biological aging process has the highest value and the most interactions consisting of nine genes. There are two best molecular functions, namely RNA polymerase II general transcription initiation factor binding and phosphatase binding, which include three and five target genes, respectively. A total of 10 potential cellular components were affected by both administered compounds and the best was caveola, which had three target genes.

Anticancer Activity and Safety Test

In vitro validation was conducted through anticancer activity and safety testing on T47D and Vero cells after analyzing the potential of the compound combination with network pharmacology. The results showed that the administration of myricetin and doxorubicin decreased the percentage viability of T47D cells, as presented by the graph in Figure 4.

There was no difference in the percentage viability of Vero cells when exposed to myricetin or doxorubicin. The results showed that the percentage viability of Vero cells decreased with higher concentrations of myricetin or doxorubicin, as presented in Figure 5.

The percentage viability obtained in T47D and Vero cells can be determined from IC50 and CC50 values of each compound. In this case, IC50 and CC50 of both administered compounds were calculated using SPSS version 20. According to Table II, IC50 values of myricetin and doxorubicin against T47D cells were 31.936 µg/mL and 27.39 µg/mL, respectively. CC50 values of myricetin and doxorubicin were 196.321 and 6.723 µg/mL, respectively. The higher the CC50 value, the lower the toxicity of the compounds against normal cells. All tests were performed in three replicates to ensure reproducibility and reliability of data. Statistical analysis was conducted using an independent T-test to compare cell viability between treatment groups. The significance level was set at $p > 0.05$, with the T-test results showing that myricetin and doxorubicin did not have significant differences due to IC50 values. However, CC50 values showed significant differences between myricetin and doxorubicin.

Calculating SI value in addition to the cytotoxic activity is important. SI implies that a substance only kills cancer cells but not normal

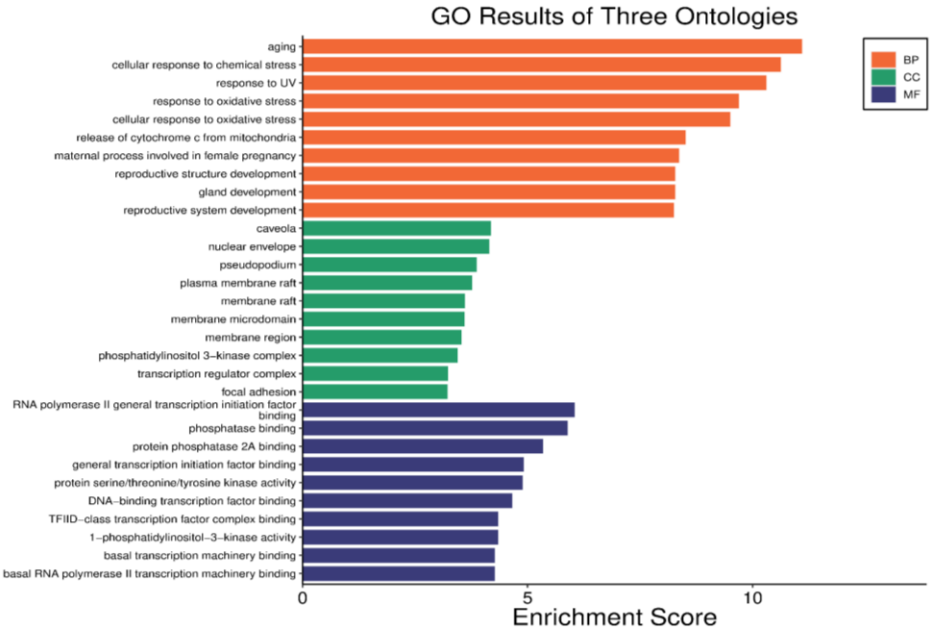

Figure 3. GO Bar Diagram for Biological Processes, Cellular Components, and Molecular Functions

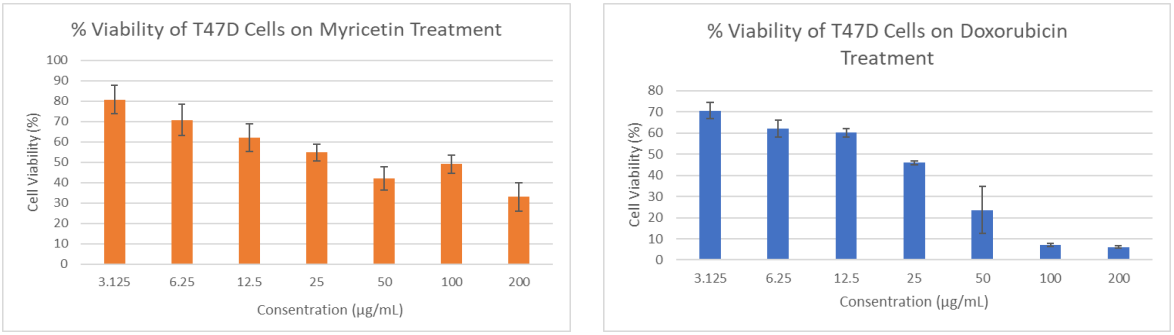

Figure 4. Bar Diagram of Percentage (%) Viability of T47D Cells with the Administration of Myricetin and Doxorubicin

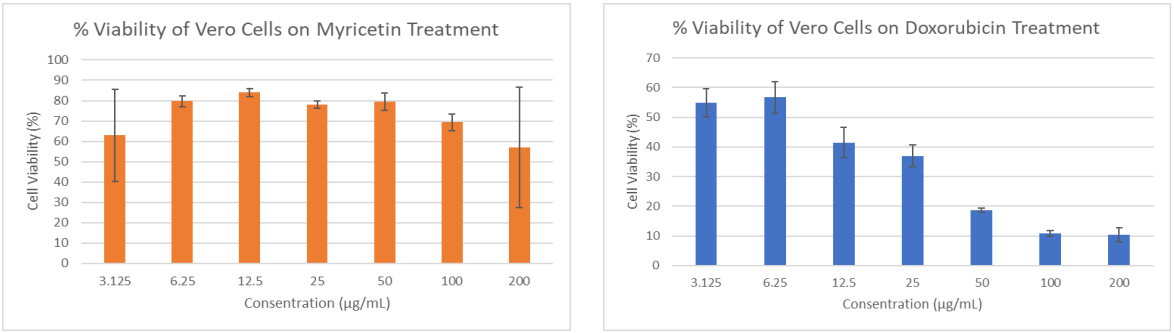

Figure 5. Bar Diagram of Percentage (%) Viability of Vero Cells with the Administration of Myricetin and Doxorubicin

cells. This is obtained from CC50 and IC50 values of each compound. Based on the calculation in Table III and the results in Table II, SI values for myricetin and doxorubicin are 6.147 and 0.245, respectively.

SI results show that myricetin is an anticancer agent capable of eliminating cancer cells only. Meanwhile, doxorubicin is an anticancer agent that can terminate both cancer and normal cells.

Table II. IC50, CC50, and T-Test Values

Samples	IC50 (µg/mL)	Normality test	Homogeneity test	T-Test
Myricetin	31.936	0.342	0.539	0.116
Doxorubicin	27.39	0.696		
Samples	**CC50 (µg/mL)**	**Normality test**	**Homogeneity test**	**T-Test**
Myricetin	196.321	0.400	0.029	0.012
Doxorubicin	6.723	0.545		

Table III. Selectivity Index

Samples	Selectivity Index	Description
Myricetin	6.147	Selective
Doxorubicin	0.245	Non-Selective

Table IV. CI of myricetin and doxorubicin

No	Concentration (µg/mL)		Cell Viability (%) ± SD	CI	Effect Category
	Myricetin	Doxorubicin			
1.	3.992	3.424	51.27 ± 13.88	0.55	Synergist
2.	7.984	3.424	33.81 ± 8.84	0.20	Powerful synergist
3.	15.986	3.424	31.27 ± 3.65	0.18	Powerful synergist
4.	31.936	3.424	32.86 ± 4.15	0.21	Powerful synergist
5.	3.992	6.48	39.41 ± 2.41	0.51	Synergist
6.	7.984	6.48	43.95 ± 10.60	0.67	Weak synergist
7.	15.986	6.48	40.77 ± 1.81	0.58	Synergist
8.	31.936	6.48	51.98 ± 4.43	1.29	Weak antagonist
9.	3.992	13.7	37.82 ± 4.17	0.98	Additives
10.	7.984	13.7	32.39 ± 7.14	0.73	Weak synergist
11.	15.986	13.7	27.32 ± 1.44	0.55	Synergist
12.	31.936	13.7	32.39 ± 0.47	0.75	Weak synergist
13.	3.992	27.39	31.80 ± 3.99	1.41	Weak antagonist
14.	7.984	27.39	32.68 ± 2.56	1.48	Antagonist
15.	15.986	27.39	28.79 ± 1.35	1.20	Weak antagonist
16.	31.936	27.39	43.42 ± 0.54	2.76	Antagonist

Combination Test

The combination test was conducted to validate the network pharmacology results of both myricetin and doxorubicin. The concentrations used in the combination test were 16 combination doses concerning IC50 results of the single test for myricetin and doxorubicin presented in Table II. CI determines the synergistic, additive, or antagonistic effect exerted by combining two compounds. The results of CI analysis showed that three, four, three, one, two, and three doses of myricetin in combination with doxorubicin had strong synergistic, synergistic, weak synergistic, additive, antagonistic, and weak antagonistic effect, respectively (Table IV and Figure 6).

Combinations that produced strong synergistic effect were 7.984 µg/mL myricetin + 3.424 µg/mL doxorubicin, 15.986 µg/mL myricetin + 3.424 µg/mL doxorubicin, and 31.936 µg/mL myricetin + 3.424 µg/mL doxorubicin. The combination with the highest IC50 concentration was selected for the test against Vero cells. Selecting the highest concentration could measure the maximum level of damage to Vero cells for comparison with the effect on cancer cells. Lower concentrations were considered safer when the combination did not show excessive toxic effect on normal cells at high concentrations. The percentage (%) cell viability of both myricetin and doxorubicin had a value of 168.756 µg/mL (Table V).

DISCUSSION

Breast cancer is among the most common types of cases reported in women worldwide. American Cancer Society (ACS) describes this cancer as a malignant tumor that attacks breast tissue consisting of mammary glands (milk-producing glands), glandular ducts (milk ducts), and supporting tissues. Cancer causes breast cells and tissues to change into abnormal shapes and undergo uncontrolled proliferation (Rastini et al., 2019). Current treatments are often ineffective due

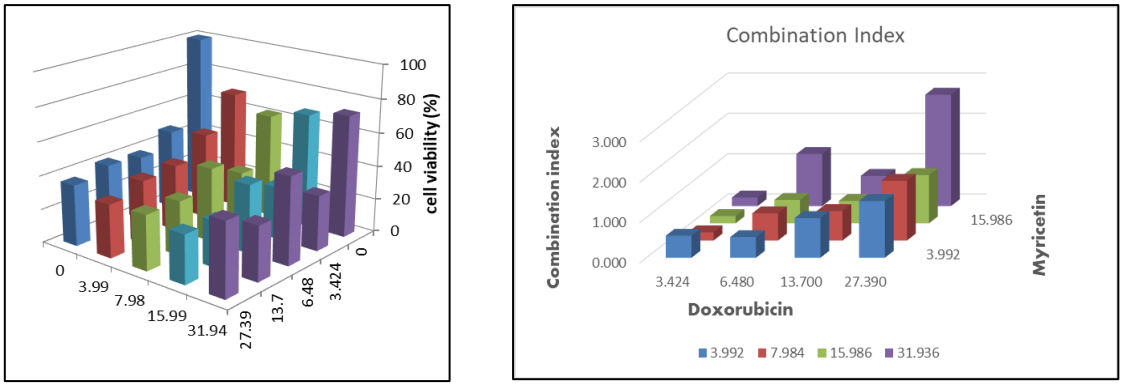

Figure 6. Percentage (%) Cell Viability and CI graph of Doxorubicin with Myricetin

Table V. Percentage (%) viability of Vero cells at the highest combination concentration

Combination	Cell Viability (%)			Mean ± SD
	1	2	3	
27.39 µg/mL Doxorubicin-31.94 µg/mL Myricetin	187.749	145.869	172.650	168.756 ± 21.210

to unspecified drug targets, resistance, and harmful side effect. Combination chemotherapy, using natural plant-based compounds with standard drugs, offers a promising method to enhance treatment efficacy and safety (Mutiah et al., 2018).

Network pharmacology was used in this study to explore how myricetin and doxorubicin might work together to treat breast cancer. By analyzing target genes with tools such as GeneCards and DisGeNET, the combination of both compounds were found to share 19 common target genes connected to breast cancer. This overlap, shown in a Venn diagram, suggested that myricetin and doxorubicin could affect similar biological pathways related to the disease. The results were further supported by PPI analysis using STRING and visualized through Cytoscape, which helped to map the interaction of the proteins in the cell. STRING analysis identified 19 proteins (called nodes) and 116 connections (or edges), with each node representing a specific protein, while the edges showed how the proteins interacted (Hu et al., 2023). The results signified that the target genes interacted closely and played important roles in the molecular processes of controlling breast cancer. Further analysis using Cytoscape provided a visual map of the interactions, showing 23 nodes and 59 edges. The map showed that the relationships between the targets tended to have clinical relevance and could help guide the development of combination treatments. In Cytoscape visualization, the nodes represented target genes, and the lines (edges) between denoted interactions of the genes (Xiang et al., 2020).

KEGG pathway analysis (Figure 2) helped identify the cellular signaling pathways influenced by both myricetin and doxorubicin. The results showed that many affected pathways were connected to apoptosis or programmed cell death. These included protein handling in ER, calcium, intrinsic, extrinsic, MAPK, PI3K-AKT, NF-kB, and p53 signaling pathways, suggesting that a combination of the compounds might work by targeting multiple mechanisms included in cancer cell death. The result was consistent with the study of Javed et al. (2022) which reported myricetin with the ability to mediate pathways such as PI3K/AKT and NF-kB contributing to cancer prevention and treatment. Apoptosis removes damaged, infected, or abnormal cells, and is essential for a healthy immune system, proper development, maintaining balance in the body, and ensuring normal cell renewal (Yanumula & Cusick, 2023). The process of apoptosis is important for eliminating damaged, old, or potentially harmful cells, such as cancerous or virus-infected types.

Protein processing in ER includes steps that help newly synthesized proteins fold and function properly. The calcium signaling pathway plays a key role in many important biological activities and is capable of triggering ROS production in the mitochondria, which affects the response of cells to stress or damage (Wu et al., 2021). The extrinsic pathway, often targeted by PARP1, triggers apoptosis through interactions between cell surface receptors and specific signals, such as

those from TNF family (Jan & Chaudhry, 2019). PARP1 is a gene affected by both myricetin and doxorubicin while showing connections to triple-negative, stage IV, ER-positive, and advanced breast cancer. The enzyme PARP1 plays a crucial role in various cellular functions, such as regulating gene activity (transcription), managing cell death (apoptosis), and helping the cell respond to DNA damage (Rose et al., 2020).

The intrinsic pathway is a type of apoptosis signaling primarily regulated by the mitochondria and is activated in response to different types of stress, such as oxidative stress, exposure to radiation, or treatment with toxic drugs. This is often targeted by BCL-2, an antiapoptotic protein for preventing cytochrome c release (Jan & Chaudhry, 2019). BCL-2 is a gene targeted by myricetin and doxorubicin, as well as associated with several diseases, including ER-positive and invasive breast cancer. Meanwhile, MAPK pathway helps regulate important cellular processes such as growth, differentiation, and response to stress (Park & Baek, 2022). The target gene in this pathway is ERK, a subtype of MAPK, including the target genes of myricetin and doxorubicin known as MAPK1 and MAPK3 associated with ER-Negative Breast Cancer.

PI3K-AKT pathway controls important cellular functions such as metabolism, growth, survival, and cell division. Furthermore, PI3K-AKT works by transmitting signals from outside the cell through a chain reaction started by PI3K enzyme. PI3K is a lipid kinase often included in cancer development and tumor growth. Downstream portion of the pathway contains AKT enzymes called serine/threonine kinases in the form of AKT1, AKT2, and AKT3. These proteins are essential for managing how cells use glucose, survive, grow, and multiply (Nunnery & Mayer, 2020). NF-kB is a signaling pathway that participates in inflammatory responses and mediates cell growth or death (Javed et al., 2022). p53 signaling pathway is controlled by p53 protein, which acts similarly to a master switch on several important genes. p53 is crucial in controlling the cell cycle, triggering apoptosis when needed, and keeping DNA stable (Wang et al., 2023).

GO analysis showed how myricetin and doxorubicin potentially treat breast cancer by influencing various biological processes, molecular functions, and cellular components. The analysis of cellular components showed the relation of the compounds to receptors, aiding in the identification of potential therapeutic target genes (Khoirunnisa et al., 2024). The biological aging process has the highest value and the most interactions consisting of nine genes (Figure 3A). Aging is a series of interrelated processes referred to as the pillars of aging. The most important mechanisms connecting cancer and aging include cellular senescence, which is a state of cells undergoing terminal growth arrest (Sedrak & Cohen, 2023). There are two best molecular functions, namely RNA polymerase II general transcription initiation factor binding and phosphatase binding, which include three and five target genes, respectively. A total of 10 potential cellular components were affected by the two administered compounds (Figure 3D) and the best was caveola, which had three target genes.

The network pharmacology results offer insights into the potential anticancer mechanisms of myricetin and doxorubicin by showing the interactions with multiple cancer-related targets. These were validated through in vitro anticancer activity testing on T47D cells and safety testing on Vero cells using myricetin-doxorubicin combination. The results showed that myricetin and doxorubicin had cytotoxic activity against T47D cancer cells, with respective IC50 values of 31.936 µg/mL and 27.39 µg/mL, signifying moderate anticancer potential (Table II). The smaller IC50 value of doxorubicin than myricetin suggested a higher cytotoxicity potential. The CC50 value (196.321 µg/mL) of myricetin higher than 6.723 µg/mL obtained for doxorubicin signified that myricetin was safer against normal cells, as evidenced by SI value above three. This observation was due to the criteria stated by Praying (2008), where compounds with SI > 3 were categorized as selective against cancer cells (Rollando & Prilianti, 2017).

Combining myricetin and doxorubicin compounds works collectively to enhance the effect produced across different doses, suggesting that the combination can be more effective than using only one. This type of teamwork, called synergism, occurs when multiple substances enhance each other to produce a stronger total effect than a single substance (Pezzani et al., 2019). Myricetin significantly enhanced the effectiveness of doxorubicin, potentially allowing for lower doxorubicin doses and reduced side effect. The synergy of both compounds appears promising, as observed through interactions with key signaling pathways (Intrinsic, PI3K-AKT, and p53) crucial for cancer cell apoptosis and proliferation. Specifically, the combination maintained high viability in normal Vero cells, showing a safer treatment profile (Nurdiani et al., 2023). The viability assessment found that combining the two compounds at the highest concentration did not produce toxic effect on normal cells, leading to

being considered safer at low concentrations. The results supported the importance of combination strategies in cancer treatment to increase efficacy while minimizing side effect.

CONCLUSION

In conclusion, the results of the network pharmacology analysis showed that myricetin combination with doxorubicin affected 19 genes participating in breast cancer, among which eight targets were found in important apoptosis signaling pathways. These included Protein Processing in ER, Calcium, Intrinsic, Extrinsic, MAPK, PI3K-AKT, NF-kB, and P53 signaling pathways. According to the in vitro assay, the combination of 7.984 µg/mL myricetin + 3.424 µg/mL doxorubicin, 15.986 µg/mL myricetin + 3.424 µg/mL doxorubicin, and 31.936 µg/mL myricetin + 3.424 µg/mL doxorubicin produced a strong synergistic effect.

ACKNOWLEDGEMENT

We would like to thank the Research and Community Service Unit (UPPM) of UIN Maulana Malik Ibrahim Malang for providing funding through the collaborative lecturer research program in 2024.

REFERENCES

Adams, C. J., Kopp, M. C., Larburu, N., Nowak, P. R., & Ali, M. M. (2019). Structure and molecular mechanism of ER stress signaling by the unfolded protein response signal activator IRE1, *Frontiers in Molecular Biosciences.*, 6:11. DOI: 10.3389/fmolb.2019.00011

Alkabban, F., M., Ferguson, T., & Menon, G. (2024). *Breast Cancer*. 2024 Feb 25. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 29493913.

Gusungi, D. E., Maarisit, W., Hariyadi, H., & Potalangi, N. O. (2020). Studi Aktivitas Antioksidan dan Antikanker Payudara (MCF-7) Ekstrak Etanol Daun Benalu Langsat *Dendrophthoe pentandra. Biofarmasetikal Tropis*, *3*(1), 166–174. https://doi.org/10.55724/j.biofar.trop.v3i1.274

Hu, M., Yan, H., Li, H., Feng, Y., Sun, W., Ren, Y., Ma, L., Zeng, W., Huang, F., Jiang, Z., & Dong, H. (2023). Use of network pharmacology and molecular docking to explore the mechanism of action of curcuma in the treatment of osteosarcoma. *Scientific Reports*, *13*(1), 9569. https://doi.org/10.1038/s41598-023-36687-z

Jan, R., & Chaudhry, G.-S. (2019). Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. *Advanced Pharmaceutical Bulletin*, *9*(2), 205–218. https://doi.org/10.15171/apb.2019.024

Javed, Z., Khan, K., Herrera-Bravo, J., Naeem, S., Iqbal, M. J., Raza, Q., Sadia, H., Raza, S., Bhinder, M., Calina, D., Sharifi-Rad, J., & Cho, W. C. (2022). Myricetin: Targeting signaling networks in cancer and its implication in chemotherapy. *Cancer Cell International*, *22*(1), 239. https://doi.org/10.1186/s12935-022-02663-2

Khoirunnisa, A., Jamil, A. S., & Muchlisin, M. A. (2024). Network Pharmacology Analysis of Secondary Metabolites of Ciplukan *(Physalis angulata L.)* Against Lung Cancer. *Majalah Farmaseutik*, *20*(2), 282. https://doi.org/10.22146/farmaseutik.v20i2.96275

Mutiah, R., Suryadinata, A., & Nurani, P. S. (2018). Uji Sitotoksik Kommbinasi Cisplatin Dengan Ekstrak Etanol Benalu Alpukat *(Dendrophthoe pentandra (L)* Miq.) pada Sel Hela. *Majalah Kesehatan*, *5*(3), 133–143. https://doi.org/10.21776/ub.majalahkesehatan.005.03.2

Nunnery, S. E., & Mayer, I. A. (2020). Targeting the PI3K/AKT/mTOR Pathway in Hormone-Positive Breast Cancer. *Drugs*, *80*(16), 1685–1697. https://doi.org/10.1007/s40265-020-01394-w

Nurdiani, E., Masriani, M., Rasmawan, R., Muharini, R., & Sartika, R. P. (2023). Sitotoksisitas dan Selektivitas Fraksi Kayu Batang Simpur Air *(Dillenia suffruticosa (Griff.) Martelli)* Terhadap Sel Kanker Payudara. *Al-Kauniyah: Jurnal Biologi*, *17*(1), 190–200. https://doi.org/10.15408/kauniyah.v17i1.31299

Park, H.-B., & Baek, K.-H. (2022). E3 ligases and deubiquitinating enzymes regulating the MAPK signaling pathway in cancers. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1877*(3), 188736. https://doi.org/10.1016/j.bbcan.2022.188736

Pezzani, R., Salehi, B., Vitalini, S., Iriti, M., Zuñiga, F., Sharifi-Rad, J., Martorell, M., & Martins, N. (2019). Synergistic Effects of Plant Derivatives and Conventional Chemotherapeutic Agents: An Update on the Cancer Perspective. *Medicina*, *55*(4), 110. https://doi.org/10.3390/medicina55040110

Priyantono, B. T. P., & Subarnas, A. (2021). Tumbuhan Herbal Sebagai Terapi Komplemen Kanker Paru. *19*.

Rahmani, A., Almatroudi, A., Allemailem, K., Alwanian, W., Alharbi, B., Alrumaihi, F., Khan, A., & Almatroodi, S. (2023). Myricetin: A Significant Emphasis on Its Anticancer Potential via the Modulation of Inflammation and Signal Transduction Pathways. *International Journal of Molecular Sciences*, *24*(11), 9665. https://doi.org/10.3390/ijms24119665

Rastini, M. B. O., Giantari, N. K. M., Adnyani, K. D., & Laksmiani, N. P. L. (2019). Moleculer Docking Aktivitas Antikanker Dari Kuersetin Terhadap Kanker Payudara Secara In Silico. *Jurnal Kimia*, 180. https://doi.org/10.24843/JCHEM.2019.v13.i02.p09

Rollando, R., & Prilianti, K. R. (2017). Fraksi Etil Asetat Kulit Batang Faloak *(Sterculia quadrifida R.Br)* Menginduksi Apoptossis Dan Siklus Sel Pada Sel Kanker Payudara T47D. *Journal of Pharmaceutical Sciences and Community*, *14*(1), 1–14. https://doi.org/10.24071/jpsc.141557

Rose, M., Burgess, J. T., O'Byrne, K., Richard, D. J., & Bolderson, E. (2020). PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. *Frontiers in Cell and Developmental Biology*, *8*, 564601. https://doi.org/10.3389/fcell.2020.564601

Sedrak, M. S., & Cohen, H. J. (2023). The Aging–Cancer Cycle: Mechanisms and Opportunities for Intervention. *The Journals of Gerontology: Series A*, *78*(7), 1234–1238. https://doi.org/10.1093/gerona/glac247

Song, X., Tan, L., Wang, M., Ren, C., Guo, C., Yang, B., Ren, Y., Cao, Z., Li, Y., & Pei, J. (2021). Myricetin: A review of the most recent research. *Biomedicine & Pharmacotherapy*, *134*, 111017. https://doi.org/10.1016/j.biopha.2020.111017

Suryaningsih, E., & Sukaca, B. E. (2009). Kupas tuntas kanker payudara. Paradigma Indonesia.

Wang, H., Guo, M., Wei, H., & Chen, Y. (2023). Targeting p53 pathways: Mechanisms, structures and advances in therapy. *Signal Transduction and Targeted Therapy*, *8*(1), 92. https://doi.org/10.1038/s41392-023-01347-1

Wu, L., Lian, W., & Zhao, L. (2021). Calcium signaling in cancer progression and therapy. *The FEBS Journal*, *288*(21), 6187–6205. https://doi.org/10.1111/febs.16133

Xiang, G., Ji, J., Feng, Z., Hou, X., Luo, Y., & Mei, Z. (2020). A network pharmacology approach to explore the potential targets underlying the effect of sinomenine on rheumatoid arthritis. *International Immunopharmacology*, *80*, 106201. https://doi.org/10.1016/j.intimp.2020.106201

Yanumula, A., & Cusick, J. K. (2023). *Biochemistry, Extrinsic Pathway of Apoptosis*. https://www.ncbi.nlm.nih.gov/books/NBK560811/

Yudistira, A. (2017). Uji Aktivitas Anti Kanker Payudara Ekstrak Daun Sesewanua *(Clerodendron squmatum Vahl.)* Terhadap Sel Kanker Payudara T47D. *6*(2). https://doi.org/10.35799/pha.6.2017.16420