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Evaluation of Anticancer Activity and Mechanism of Action of Myricetin on HeLa, T47D, and Vero Cells: Comparative Analysis with Cisplatin and Doxorubicin

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Cervical and breast cancers are two of the most common cancer affecting women. In Indonesia, there are 65,858 cases of breast cancer and 36,633 cases of cervical cancer have been recorded. Chemotherapy, using agents such as cisplatin and doxorubicin, is one of the main cancer therapies and works by targeting cancer cells. However, this therapy lacks selectivity and damages normal cells, leading to adverse side effects. An alternative chemopreventive treatment is Myricetin, a compound predicted to potentially target VEGF, a critical factor in angiogenesis, making it a promising anticancer agent. This study aims to evaluate the safety of Myricetin on Vero cells (normal cells), assess its anticancer activity on T47D and HeLa cells, and predict its mechanism of action. The anticancer activity was evaluated using the Microculture Tetrazolium Technique (MTT) assay on HeLa, T47D, and Vero cells. VEGF receptors were identified through a Network Pharmacology approach. The study also involved the Molecular Docking of Myricetin, cisplatin, and doxorubicin compounds with the 5DXH receptor. The results showed that Myricetin exhibited high cytotoxic activity against HeLa and T47D cells, with IC50 values of 22.70 μg/mL and 51.43 μg/mL, respectively, while demonstrating significantly lower cytotoxicity against Vero cells, with a CC50 value of 1445.2 μg/mL. In comparison, the CC50 values for cisplatin and doxorubicin against Vero cells were 6.53 μg/mL and 13.76 μg/mL, respectively, indicating that Myricetin is considerably less toxic to normal cells. Myricetin's Selectivity Index (SI) was 63.64 for HeLa cells and 28.09 for T47D cells, demonstrating superior selectivity compared to cisplatin and doxorubicin. These findings suggest that Myricetin has promising anticancer potential with a better safety profile than conventional chemotherapeutic agents.

Keywords: Cisplatin; Doxorubicin; HeLa cells; Myricetin compound; T47D cells; VEGF; Vero cells.

Cancer is one of the leading causes of death worldwide, characterized by the uncontrolled growth of abnormal cells that can invade healthy tissues and spread to other organs.[1] Breast cancer and cervical cancer are among the most prevalent and pose significant threats to women's health. According to Globocan 2020 data, Indonesia recorded 65,858 new cases of breast cancer and 36,633 cases of cervical cancer.[2] These high case numbers highlight that cancer remains a serious challenge both nationally and globally.[3] The etiologies of these cancers differ and encompass

genetic variables, including BRCA1 and BRCA2 gene mutations in breast cancer and HPV infections (types 16 and 18) in cervical cancer.[3] Additionally, unhealthy lifestyles, exposure to carcinogenic substances, obesity, and aging are significant factors that increase cancer risk.

Contemporary cancer treatments, such as chemotherapy, radiation, and hormone therapy, seek to inhibit or eradicate cancer cells. However, these treatments are often non-selective, which target cancer cells and harms normal cells. This can lead to adverse side effects such as nausea, hair loss, and damage to vital organs. Consequently, there is a need for alternative therapies that are both effective at killing cancer cells and exhibit lower toxicity to normal cells. Natural compounds like Myricetin have garnered significant attention in this context due to their relatively safe properties and substantial potential as anticancer agents.

Myricetin is a flavonoid compound with diverse pharmacological activities, including anticancer, antioxidant, and anti-inflammatory effects.[4] Previous studies have shown that Myricetin can inhibit the Vascular Endothelial Growth Factor (VEGF) signaling pathway, a critical angiogenic factor that promotes tumor growth.[5] By targeting VEGF, Myricetin reduces the formation of new blood vessels essential for delivering oxygen and nutrients to tumors, potentially inhibiting cancer growth and metastasis. Moreover, Myricetin has demonstrated the ability to induce apoptosis and inhibit the cell cycle, further establishing its potential as a candidate for more selective and safer cancer therapies.

Several clinical trials have explored the role of flavonoids in the prevention and treatment of cancer, particularly through antioxidant, anti-inflammatory, and modulating the gut microbiota. An ongoing NCT03959618 clinical trial in France aims to evaluate the role of *Desmodium adscendens*, a source of flavonoids and other polyphenols, as an adjunct therapy in standard intravenous chemotherapy for breast cancer patients.[6] Meanwhile, the NCT03615599 large-scale study, involving about 96,000 participants, found that adherence to a plant-based diet was associated with a lower risk of breast cancer.[7] High consumption of fruits, vegetables, whole grains, nuts, and legumes is associated with a reduced risk of breast cancer. In contrast, a high intake of animal products and processed foods increases the risk.[6]

The study aimed to evaluate the anticancer activity of Myricetin in cervical cancer cells (HeLa) and breast cancer cells (T47D) in vitro, as well as to compare its effectiveness with cisplatin and doxorubicin, two commonly used chemotherapy agents. The study also employed Vero cells as a model to evaluate the toxicity of Myricetin in normal cells. Furthermore, an insilico method, including molecular docking and network pharmacology, was utilized to examine the mechanism by which Myricetin inhibits VEGF. The research methodology entailed evaluating the anticancer efficacy of Myricetin using the Microculture Tetrazolium Technique Assay (MTT Assay) on HeLa, T47D, and Vero cells to ascertain IC50 and CC50 values, indicative of cytotoxic capability and selectivity. The VEGF target protein was identified through network pharmacology analysis, and molecular docking was used to predict Myricetin's molecular interaction with the VEGF receptor. This research is expected to contribute to developing safer and selective natural ingredients-based anticancer therapies to reduce the adverse effects of current conventional therapies.

MATERIAL AND METHODS

This study aims to evaluate the anticancer activity of the Myricetin compound in cervical cancer (HeLa) and breast cancer (T47D) cells in vitro and compare its effectiveness with cisplatin and doxorubicin. In addition, Myricetin's toxicity to normal cells (Vero) was also evaluated. In silico approaches, such as network pharmacology and molecular docking, are used to study Myricetin's interactions with VEGF receptors, which are key molecular targets.

Materials Used

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Source of Used Cells

HeLa and T47D cells were obtained from the American Type Culture Collection (ATCC, USA) as a model for cervical cancer and breast cancer. Meanwhile, Vero cells as a normal cell model were obtained from African Green Monkey Kidney (ATCC CCL-81, USA). All cells are cultured in suitable media until they reach a density of 70-80% before being used in research.

Cytotoxicity Test

The cytotoxicity test has been performed to examine Myricetin's ability to prevent the development of HeLa and T47D cancer cells, as well as its toxicity to Vero's normal cells. This test refers to and modifies from Foot et al., and Ghasemi et al. [8,9] Cells that reached optimal density are inoculated into 96-well microplates with a concentration of 10t cells/well. After incubation for 24 hours, cells were treated with Myricetin, cisplatin, and doxorubicin at various concentrations (3.125, 6.25, 12.5, 25, 50, 100, 200 ìg/mL) for 48 hours. After treatment, MTT (5 mg/mL) is added to each well, and incubation continues for 4 hours. The formed formazan crystals were then dissolved using DMSO, and the absorbance was measured at a wavelength of 570 nm using an ELISA Reader. The absorbance value is used to calculate the IC_{50} and CC_{50} values, which reflect Myricetin's cytotoxic activity and selectivity.

Network Pharmacology Analysis

Network pharmacology analysis was conducted to identify Myricetin's molecular targets and elucidate its action mechanism in inhibiting tumor growth. The GeneCards database (https://www.genecards.org) was utilized to retrieve target genes associated with Myricetin activity, while the DisGeNET database (https://www.disgenet.org/) was used to obtain target genes linked to cervical and breast cancers. The STRING database (https://string-db.org) was also employed to construct a protein-protein interaction (PPI) network.[10] The PPI network was visualized with a minimum interaction confidence threshold of 0.400. This analysis provided insights into the relationship between Myricetin and VEGF in angiogenesis.

Molecular Docking

Molecular docking was conducted to predict the interaction between Myricetin and the VEGF receptor, the primary target in this study.[10] The three-dimensional structures of Myricetin, cisplatin, and doxorubicin were generated using ChemBio3D Ultra software, with the ligand structures optimized using the minimal energy method to ensure stable conformations. The VEGF receptor structure (PDB ID: 5DXH) was obtained from the Protein Data Bank and prepared by removing water molecules and native ligands to define the binding site. The docking process used Molegro Virtual Docker (MVD) with default parameter settings to identify interactions at VEGF-targeted sites. Docking analysis evaluated binding energy and identified specific interactions between Myricetin and critical amino acids in the VEGF receptor. The docking results were compared with those of cisplatin and doxorubicin to assess the relative efficacy of Myricetin as a VEGF inhibitor.

Data Analysis

The absorbance data obtained using an ELISA reader is converted into cell viability (% of living cells) with the formula.[8]

Cell Viability = (Absorbance of treatment-Absorbance of media control)/(Absorbance of cell control –Absorbance of media control) x 100%

The calculation of cell viability was used to calculate the IC_{50} value (the concentration that causes the death of 50% of the cell population) for HeLa and T47D cells, as well as the CC_{50} value (the concentration that reduces cell viability by 50%) for Vero cells. IC_{50} values > 500 ìg/ml indicate compounds with weak cytotoxic activity, while IC_{50}/ CC_{50} values < 500 ìg/ml indicate high cytotoxic activity.[11] Data analysis from molecular docking was carried out by considering the rerank score value and amino acid residue interaction as parameters to assess the strength of the compound interaction against the target protein.

RESULTS

Myricetin Compound Toxicity Test on Hela Cells, T47D, and Vero Cells via MTT Assay

Table 1 shows a decrease in cell viability of HeLa cells as the concentration (ppm) of Myricetin, cisplatin, and doxorubicin increases, although the changes are not statistically significant. At the highest concentration of Myricetin (200 ìg/mL), the average cell viability is 20.23%, while at the

Table 1. Average percent cell viability and IC_{50} value compound *Myricetin* in HeLa cells

No	Sample	Average % Viability of HeLa cells ± SD At test concentration (µg/mL)							IC_{50}(µg/mL) ±SD
		3.125	6.25	12.5	25	50	100	200	
1	*Myricetin*	65.66 ±2.82	70.11± 0.82	71.88± 3.58	51.76± 4.06	36.99± 0.54	25.64±4.21	20.32± 0.72	22.70±1.20
2	Cisplatin	132.76±3.39	110.57±1.0	84.85±2.06	39.47±4.46	25.47±1.34	16.00±0.58	17.38±1.07	28.96±3.33
3	Doxorubicin	36.65±1.83	28.97±0.68	28.11±1.99	24.36±1.97	7.25±1.4	0.89±0.51	2.78±0.18	1.91±0.17

Table 2. Average percent cell viability and IC50 value compound Myricetin in T47D cells

No	Sample	Average % Viability of T47D cells ± SD At test concentration (µg/mL)							IC_{50}(µg/mL) ±SD
		3.125	6.25	12.5	25	50	100	200	
1	Myricetin	75.78±1.12	72.16±1.09	58.54±0.22	57.70±0.82	42.0±1.24	50.22±0.30	38.03±0.45	51.43±2.63
2	Cisplatin	117.3±1.28	86.47±1.49	56.76±2.72	26.3±1.42	20.3±1.42	15.99±1.85	14.26±1.85	17.9±1.06
3	Doxorubicin	53.04±0.81	53.53±1.25	37.62±1.55	36.79±0.93	17.99±0.68	9.65±1.4	10.62±1.39	5.67±0.15

Table 3. Average percent cell viability and CC50 value compound Myricetin in Vero cells

No	Sample	Average % Viability of Vero cells ± SD At test concentration (µg/mL)							IC_{50}(µg/mL) ±SD
		3.125	6.25	12.5	25	50	100	200	
1	Myricetin	53.68±1.25	75.82±2.56	80.87±3.4	74.35±1.78	76.35±0.81	65.02±2.43	37.19±1.99	1445.2±69.46
2	Cisplatin	78.47±1.26	46.67±1.71	21.72±1.45	22.56±1.7	21.82±0.83	16.67±0.75	9.89±0.89	6.53±0.28
3	Doxorubicin	69.48±1.12	61.46±2.01	60.72±1.93	46.75±0.84	31.84±1.78	8.61±0.7	7.47±0.53	13.76±1.02

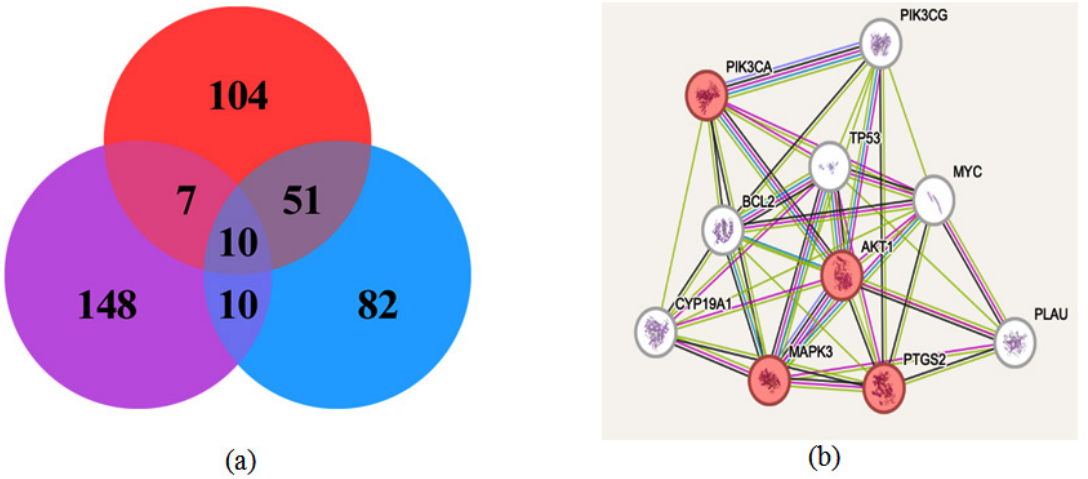
lowest concentration (3.125 ìg/mL), the average cell viability is 65.66%. Compounds with high cytotoxic activity have IC_{50} values of < 500 ìg/mL, whereas compounds with weak cytotoxic activity have IC_{50} values > 500 ìg/mL4.Based on the IC_{50} values calculated in Table 1, Myricetin and cisplatin have IC_{50} values of 22.70 ìg/mL and 28.96 ìg/mL, respectively. This indicates that both Myricetin and cisplatin exhibit high cytotoxic activity against HeLa cells. In contrast, the IC_{50} value for doxorubicin against HeLa cells is 1.91 ìg/mL, suggesting that doxorubicin has a higher activity against HeLa cells. A lower IC50 value indicates a stronger ability of the compound to inhibit the biological activity of its target. Therefore, compounds with lower IC_{50} values are more effective inhibitors than those with higher IC_{50} values.

The viability of T47D cells decreased with the increase in the anti-cancer compound concentration (Table 2). Myricetin compound has an IC_{50} value of 51.43 ìg/mL, which indicates that Myricetin compound has high cytotoxic activity against T47D cells. On the other hand, the IC_{50} obtained on the positive control of cisplatin and doxorubicin against T47D cells was 17.9 ìg/mL and 5.67 ìg/mL. Cisplatin and doxorubicin have lower IC_{50} values, indicating that both compounds have higher activity against T47D cells.

The treatment with Myricetin at a concentration of 200 ìg/mL resulted in a lower average cell viability percentage of 37.19%. In contrast, treatment with Myricetin at the lowest concentration of 31.25 ìg/mL produced a higher average cell viability percentage of 53.68%. For cisplatin and doxorubicin at a concentration of 200 ìg/mL, cell viability percentages were significantly lower at 9.89% and 7.47%, respectively. When treated with cisplatin and doxorubicin at a concentration of 6.125 ìg/mL, Vero cells' average cell viability percentages were 78.47% and 69.48%, respectively. Myricetin exhibited a CC_{50} value of 1445.2 ìg/mL against Vero cells, whereas the CC_{50} values for cisplatin and doxorubicin were 6.53 ìg/mL and 13.76 ìg/mL, respectively. A higher CC_{50} value indicates a lower toxicity of a sample. The comparison between Myricetin and the positive controls (cisplatin and doxorubicin) revealed a significant difference in CC_{50} values, indicating that Myricetin is safer for normal cells compared to cisplatin and doxorubicin.

Network Pharmacology Analysis of Target Gene and VEGF Signaling Pathway

The determination of target genes using the GeneCards database identified 175 target genes associated with the Myricetin compound. The DisGeNET database revealed 154 target genes for cervical cancer and 172 for breast cancer. To predict



(a) (b)

Fig. 1. (a) Venn diagram showing overlapping target genes associated with Myricetin-related pathways and diseases. (b) Protein-protein interaction network highlighting key molecular targets of Myricetin in the VEGF signaling pathway, where node colors indicate degree of interaction and edge colors represent different types of molecular associations

the target genes of Myricetin in cervical and breast cancers, a Venn diagram analysis was performed to identify overlapping genes between the compound and the diseases, resulting in 10 shared target genes (Figure 1(a)).

Protein-protein interactions (PPIs) using STRING (https;//STRING-db.org/) aim to determine the relationship between different types of target genes in Myricetin compounds that have a role in the treatment pathway of breast and cervical cancer. The results showed that Myricetin compounds have the potential to interact with certain proteins involved in biological processes relevant to the treatment of cervical cancer and

Table 4. 5DXH receptor validation results

Parameters	5H2_1101[A] Replication I	Replication II	Replication III	Average ± SD
RMSD Value (Å)	0.97	0.82	0.82	0.87 ± 0.086
Rerank Score (kcal/mol)	-105.989	-102.324	-99.4672	-102.5934 ±3.269

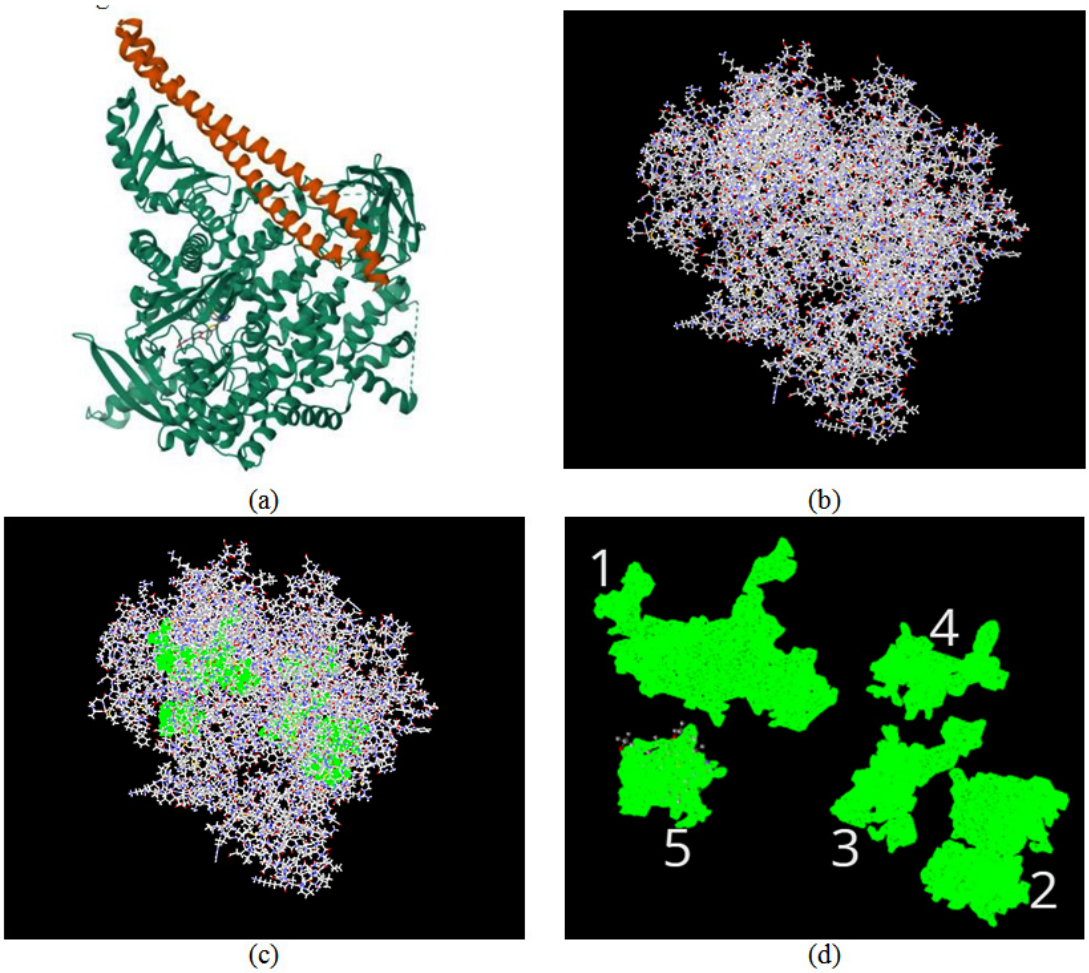


Fig. 2. (a) Results of downloading the target protein of VEGF PDB ID 5DXH (RCSB PDB, 2024); (b) 5DXH receptors; (c) Cavity detection results in the 5DXH receptor; and (d) Cavity 1 Vol = 1193.47 Surface = 1931.52, Cavity 2 Vol = 583.168 Surface = 1598.72, Cavity 3 Vol = 415.232 Surface = 1194.24, Cavity 4 Vol = 288.768 Surface = 915.2, and Cavity 5 Vol = 263.68 Surface = 798.72

breast cancer. There are 10 target genes of the compounds that have the most potential for cervical cancer and breast cancer based on the cluster results of the STRING indicated by Figure 1 (b), namely PIK3CA, PIK3CG, TP53, MYC, BCL2, CYP19A1, AKT1, PLAU, PTGS2, and MAPK3 by showing the presence of a disease pathway from cervical cancer and breast cancer, namely the VEGF signaling pathway.

5DXH receptor selection and validation

The selection of receptors in molecular docking is carried out based on several special parameters, including the identity of 100% and the selection of receptors with a resolution below 3 Å. This is done to provide a very detailed picture of the structure of the protein, including the position of the atoms and the configuration of the space. This

Table 5. Results of docking Myricetin compounds and comparator compounds (Cisplatin, and Doxorubicin) with 5DXH receptors

Compound	Score Parameters	Replication (kcal/mol)			Average ± SD
		Replication I	Replication II	Replication III	
Myricetin	*Rerank score*	-80.5759	-80.4839	-80.1474	-80.402±0.22
Cisplatin	*Rerank score*	-42.3352	-43.366	-42.3375	-42.679±0.59
Doxorubicin	*Rerank score*	-73.3337	-79.1111	-76.1684	-76.204±2.88

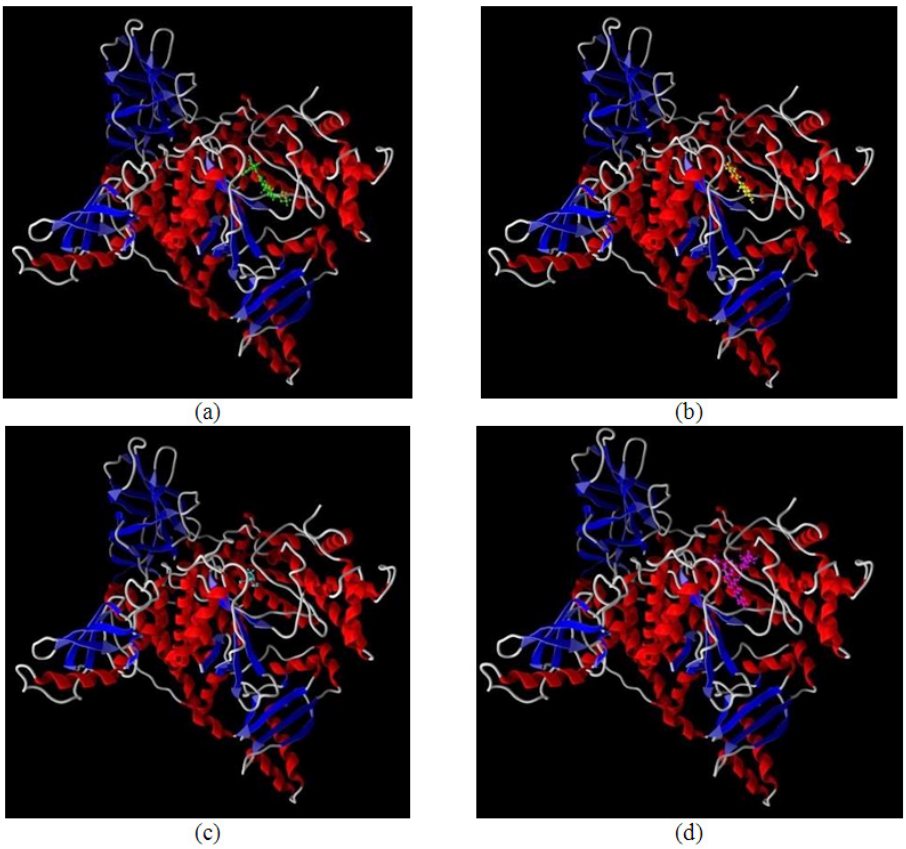


Fig. 3. The form of secondary structure results from docking compounds with receptors (a) Ligan Nativity, (b) Myricetin Compound, (c) Cisplatin, and (d) Doxorubicin. The structures highlight key binding interactions. Myricetin shows distinct binding behavior compared to standard chemotherapeutic agents, suggesting a potential alternative with unique interaction properties

allows for more accurate prediction of interactions between ligands and receptors, which is essential for effective drug design.[12] From some of the parameters that have been explained, the receptors that meet the requirements are from the PI3KCA gene with the PDB code: 5DXH.

The VEGF receptor protein target is downloaded through the website (https://www.rcsb.org/structure/6m2n) in the format *pdb. Search and download of receptors based on receptor proteins that already have ligaments. The VEGF receptor protein used in the study, namely the protein with PDB ID 5DXH, is shown in Figure 2 (b). Receptors that have been downloaded via PDB with PDB ID 5DXH with native ligand 5H2_1101. The protein was reviewed using the Molegro Virtual Docking (MVD) Software shown in Figure 2 (b). The MVD program will automatically correct the proteins added to the workspace and directly add H atoms and correct if there are some residual amino acids that are wrong in both valence and charge. Figure 2 (c) and (d) shows the existence of 5 possible cavities that will interact with the 5DXH receptor. Of the five holes that interact with the 5DXH

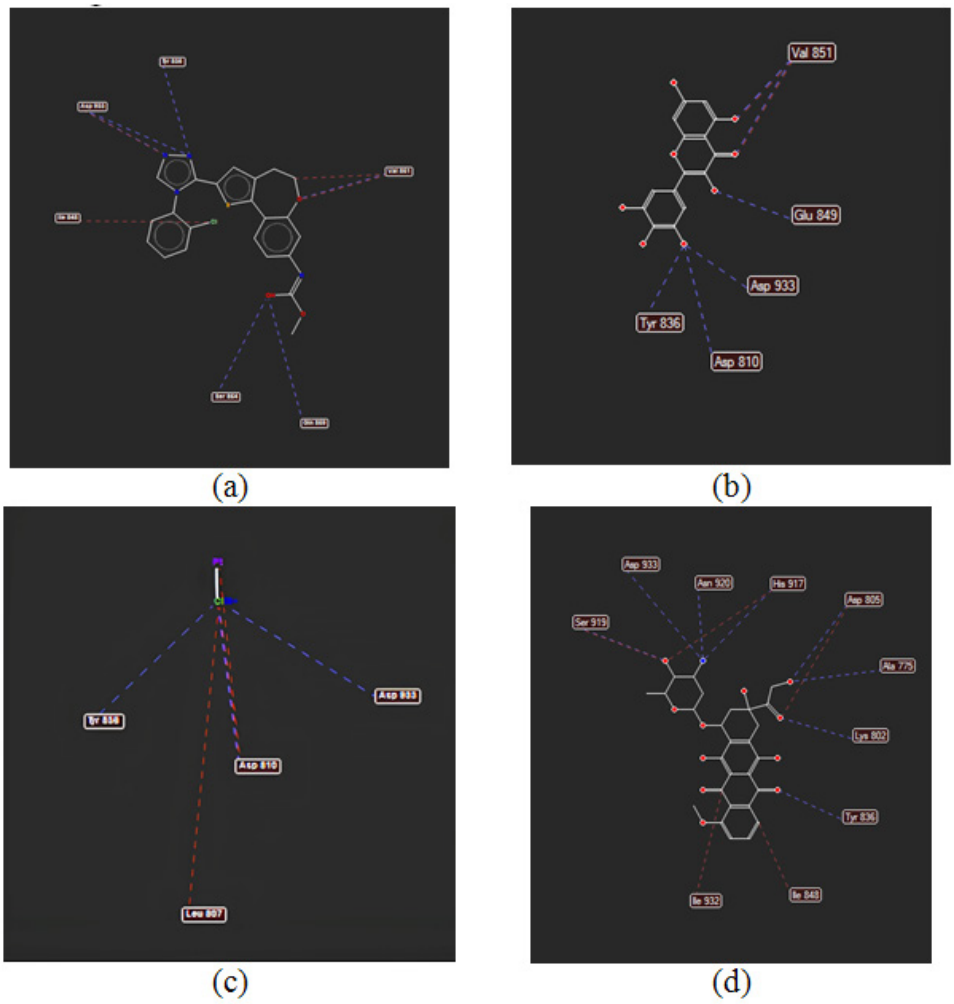


Fig. 4. Results of amino acid interaction with 5DXH receptors with (a) native ligands, (b) Myricetin compounds, (c) Cisplatin, and (d) Doxorubicin. The molecular docking analysis reveals hydrogen bonds (blue dotted lines) and steric interactions (red lines), which contribute to ligand-receptor binding. Myricetin, cisplatin, and doxorubicin exhibit hydrogen and steric interactions, whereas the native ligand also forms electrostatic bonds. The amino acid residues involved in these interactions are critical in determining the anticancer potential of Myricetin in comparison to standard chemotherapeutic drugs

receptor, the hole that interacts is cavity 5 with a volume of 263.68 $Å^3$ and a surface area of 789.72 $Å^2$.

Receptor validation was replicated three times by re-docking between the native ligand and the 5DXH target protein receptor cavity. The 5DXH receptor has 4 different proteins including 5H2_1101 [A], 5H2_1101 [B], 5H2_1101 [C], and 5H2_1101 [D]. Based on the results of receptor validation in Table 5.11, the average value of RMSD was obtained at 0.87 Å. RMSD explained the value of the distance of the atom in one conformation with the nearest atom with the same category as the atom in another. The smaller the RMSD value, the better the position of the estimated ligand, because it is closer to the original conformation.[13] These results show that the receptor validation criteria have been met, because if the standard ligand tethering results have an RMSD value of d" 2 Å, then the tethering parameters can be accepted or declared valid.[14]

Docking Myricetin, Cisplatin, and Doxorubicin Compounds at 5DXH Receptors

The docking of Myricetin, Cisplatin, and Doxorubicin compounds was carried out using Molegro Virtual Docker 6.0 software. There are 2 parameters used, namely Rerank score and hydrogen bonding of amino acid residues (H-bond). These two parameters are scores that can measure the strength of drug binding to receptors.[15] The Rerank score reflects the bond energy (total calculation of all existing bonds) needed to form a bond between the ligand and the receptor so that it can be used to predict the activity of a compound.[16]

The results of the analysis of the docking of the VEGF RECEPTOR (PDB ID:5DXH) with Myricetin compounds and comparators (cisplatin and doxorubicin) as listed in Table 5. The lowest average Rerank Score was -80,402 kcal/mol in the Myricetin test compound, while Cisplatin and Doxorubicin as comparison compounds had an average Rerank Score of -42,679 kcal/mol and 76,204 kcal/mol. The rerank score can be used to evaluate the quality of docking, predict its affinity, and look for the right ligand conformation by looking at the lowest value.[17] Based on these results, it can be said that Myricetin compounds have higher activity than comparator drugs. Test compounds that have a lower affinity value than comparator compounds are predicted to have more stable binding ability than comparator compounds.[18]

Results of Ligan Interactions with Amino Acids

The results of the interaction between the ligand and amino acids at the target PDB ID 5DXH receptor are shown in Figure 4. The amino acid interactions obtained in molecular docking using MVD software are hydrogen bonding, steric, and electrostatic interactions. The interaction between ligands and receptors using Molegro Virtual Docker software produces three types of bonds, namely hydrogen, electrostatic, and steric bonds.[19] However, the interaction between Myricetin compounds and comparator drugs, namely cisplatin and doxorubicin, with receptors only forms hydrogen and steric bonds. In Figure 4, the hydrogen bond is marked with a blue dotted line, while the steric bond is marked red. Hydrogen bonding occurs through the interaction of the H atom with the electronegative atom at a distance of 1.72–2.85 Å.[20] Amino acid residues involved in this interaction play an important role in determining the biological activity of compounds compared to comparator ligands and original ligands.[21]

DISCUSSION

Myricetin is a flavonoid found in various natural sources such as fruits, vegetables, tea, and wine. Some of the plant families with the highest myricetin content include Myricaceae, Polygonaceae, Primulaceae, Pinaceae, and Anacardiaceae.[22] The myricetin content in foods varied, with the highest concentrations found in cranberries (6,600 mg/100 g), dock (5,700 mg/100 g), sweet potato leaves (4,400 mg/100 g), and blackberries (700 mg/100 g).[22] In addition to myricetin, other phenolic compounds, such as quercetin and kaempferol, are also found in a variety of plant sources and have significant anticancer activity.[23] Polyphenols work through a variety of mechanisms, including antioxidant, anti-inflammatory, and carcinogen-metabolizing enzyme expression modulation, which can contribute to cancer prevention.[24] Thus, the consumption of foods rich in myricetin and other phenolic compounds has the potential to be a natural strategy in cancer prevention.

Myricetin compounds exhibit a higher cell viability range than the comparator drugs.

This can be attributed to the properties of Myricetin as a flavonoid, known for its cytotoxic activity against cancer cells. Feng,[25] shows that Myricetin can induce apoptosis and inhibit the proliferation of cancer cells, although its effectiveness depends on the concentration and type of cancer cells being tested. In contrast, doxorubicin is a chemotherapy agent that works by intercalating into DNA and inhibiting DNA synthesis, ultimately leading to cancer cell death. The differences in the mechanisms of action of these compounds influence their ability to kill cancer cells. Doxorubicin and cisplatin have a more direct mechanism of action in damaging cancer cell DNA, while Myricetin primarily focuses on signal modulation and apoptosis induction.

The VEGF signaling pathway (hsa04370) has a count in network value of 4 of 56, so this pathway is related to the target gene of the Myricetin compound with a total of 4 protein interactions, namely the genes PI3KCA, AKT1, MAPK3, and PTGS2. PI3KCA is a gene that encodes the PI3K enzyme, which is a crucial component in signaling pathways that regulate various cellular functions. Overactivation or mutations in PI3KCA are often found in various types of cancer, including breast and cervical cancer, which accelerate tumor growth.[26] Within the VEGF pathway, AKT1 increases the production of nitric oxide (NO) molecules, which causes blood vessels to dilate (vasodilation) and allow the formation of new blood vessels, all of which favor the growth of larger tumors.[27] In addition, MAPK3, also known as ERK1, plays a role in the MAPK pathway that regulates many important cellular functions, including cell growth and stress response. VEGF can increase PTGS2 expression, which in turn accelerates angiogenesis and worsens tumor conditions, making them more invasive and aggressive.[17] Myricetin can suppress the expression of VEGF at the transcriptional level, such as hypoxia-inducible factor-1á (HIF-1á), which is crucial for upregulating VEGF expression under hypoxic conditions, commonly found in tumors.[22] By reducing HIF-1á expression, myricetin lowers the amount of VEGF produced by cancer cells, thereby limiting the stimulation of angiogenesis.

Based on the table presented, the native ligand (5H2_A) of the 5DXH receptor binds to five amino acid residues: Asp 933, Tyr 836, Val 851, Gln 859, and Ser 854. The docking results showed that Myricetin, cisplatin, doxorubicin, and native ligands had different interaction patterns in the type of bond and the amino acid residues involved. This analysis indicates that Myricetin has the potential to be a competitive ligand compared to cisplatin and doxorubicin, which is assessed based on amino acid interactions at the protein's active site, hydrogen bond distance, and steric interactions that occur.[28] This finding correlates well with the cytotoxicity test results, which showed that Myricetin exhibited high cytotoxic activity against HeLa and T47D cells, with IC50 values of 22.70 ìg/mL and 51.43 ìg/mL, respectively, while demonstrating low cytotoxic activity against Vero cells, with a CC50 value of 1445.2 ìg/mL. Myricetin's Selectivity Index (SI) was 63.64 for HeLa cells and 28.09 for T47D cells, indicating better selectivity than cisplatin and doxorubicin. Additionally, Myricetin exhibited a significantly higher CC50 value against Vero cells (1445.2 ìg/mL) compared to cisplatin (6.53 ìg/mL) and doxorubicin (13.76 ìg/mL), suggesting that Myricetin is considerably safer for normal cells.

Myricetin compounds exhibit strong interactions with several amino acids at the active site of the 5DXH protein. For example, Myricetin interacts with Val 851, Asp 933, and Tyr 836 via relatively short-distance hydrogen bonds, such as in Asp 933 (2.75 Å) and Tyr 836 (2.46 Å). This short bond distance indicates strong hydrogen bonds, which contribute to the stability of the protein-ligand complex. In addition, Myricetin also showed stereoscopic interactions with Val 851, an amino acid that also interacts with native ligands, confirming that Myricetin can occupy the same active site as the protein's natural ligand. This indicates that Myricetin has a good affinity for the target protein.[27]

Cisplatin, which was used as a comparator, showed different interaction patterns. These compounds interact with amino acids such as Asp 810 and Asp 933 through hydrogen bonds at a distance of about 2.39-2.60 Å. Although this distance is relatively short and shows a fairly strong bond, the amount of amino acids interacting with cisplatin is less than Myricetin. This suggests that cisplatin has a lower affinity to interact with the 5DXH protein than Myricetin. Meanwhile, doxorubicin exhibits more complex interactions

involving many amino acids, such as Ser 919, His 917, and Asp 805, but with a longer bond distance (2.69-3.31 Å). This longer distance indicates that the interaction of doxorubicin with proteins is less stable compared to Myricetin and cisplatin.[26]

The steric interactions observed in this study also strengthen the results of hydrogen bond analysis. Myricetin, for example, showed significant steric interactions with Val 851 at a distance of about 2.98-3.09 Å. This interaction is similar to the native ligand, which interacts with Val 851, suggesting that Myricetin can mimic the protein's natural ligand interaction patterns. In contrast, cisplatin and doxorubicin show steric interaction patterns that tend to be more diffuse and have nothing in common with ligand natives. Cisplatin primarily interacts with Asp 810 through steric interactions over relatively longer distances, exhibiting a weaker affinity than Myricetin. Meanwhile, doxorubicin has more steric interactions with amino acids such as Ile 848 and Ile 932, but some of them differ from native ligands, which can reduce the effectiveness of specific binding to target proteins.[29]

Overall, the analysis of hydrogen and steric interactions showed that Myricetin had a better affinity for the 5DXH target protein than cisplatin and doxorubicin. This is reinforced by the interaction pattern of Myricetin, which resembles a native ligand in terms of both the amino acids involved and the bond distance. Thus, Myricetin can potentially be a new inhibitor candidate more selective against the target protein. This provides a foundation for further developing Myricetin as a complementary therapeutic agent, especially in treating diseases involving the 5DXH protein, such as cancer.[30] This study primarily relies on molecular docking and in vitro cytotoxicity assays, which, while informative, do not fully capture the complexity of in vivo conditions. The variability of cancer cell types and potential resistance mechanisms were not extensively explored, which may affect the generalizability of the findings. Additionally, the study lacks comparative analyses with other flavonoids, which could provide a broader context for Myricetin's anticancer potential. Future research should include in vivo validation and mechanistic studies to confirm Myricetin's therapeutic applicability.

CONCLUSION

Myricetin exhibits strong cytotoxic activity against HeLa (IC50: 22.70 ìg/mL) and T47D (IC50: 51.43 ìg/mL) cancer cells while showing low toxicity to normal Vero cells (CC50: 1445.2 ìg/mL). Its high selectivity index (63.64 in HeLa, 28.09 in T47D) surpasses cisplatin and doxorubicin, which have much lower selectivity. Additionally, Myricetin's strong affinity for VEGF receptors enables it to inhibit angiogenesis, highlighting its potential as a safer and more effective cancer therapy. However, the study is limited by the lack of in vivo validation and comparisons with other flavonoids, necessitating further research to confirm Myricetin's clinical applicability.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Author Contribution

Alvi Milliana: Conception, Supervision, Resources, Material, Literature Search, Writing, Critical Review; Riza Ambar Sari: Resources, Literature Search, Critical Review; Shella Al Miranda: Design, Data Collecting and/or processing, Analysis and/or Interpretation, Writing; Roihatul Mutiah: Conception, Design, Supervision, Material, Data Collecting and/or Processing, analysis, Literature Search.

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