Bioinformatics and Molecular Docking Study of Amentoflavone and 3,8-Biapigenin as Inhibitors on Cervical Cancer Proteins

Dhecella Winy Cintya Ningrum, Triana Arum Kusumaningtyas, Rifki Febriansah*, Melisa Junianandа, Sri Tasminatun, Annisa Krisridwany

Department of Pharmacy, Faculty of Medicine and Health Science, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia

Abstract

Cervical cancer maintains its second-place ranking for Indonesia's highest number of cancer cases. In 2021, there were 36,633 cases of cervical cancer in Indonesia, with a rising death rate. Commonly, chemotherapy is used to treat cervical cancer and can improve the survival chances of patients, but these therapies imply increased toxicity. Biflavonoid group compounds like amentoflavone and 3,8-Biapigenin have the potential to act as anticancer agents by modulating multiple signaling pathways. This study aims to determine the cervical anticancer potential of amentoflavone and 3,8-Biapigenin based on *in silico* study. Prediction of anticancer activity *in silico* using Prediction of Activity Spectra for Active Substances (PASS) online, followed by target protein tracing using STITCH-STRING, then receptor analysis test using Ramachandran plot. A molecular docking test was conducted to determine the binding affinity of the compound with the receptor. Based on the online PASS, the compounds as thought to have low cervical anticancer potential if tested on a laboratory scale. STAT3, EP300, CYP1A1, and AKR1C1 proteins used in this study have met the requirements of a suitable receptor for molecular docking test. The best binding affinity was obtained at the interaction of amentoflavone and STAT3 with a better docking score (-9.3 kcal/mol) than doxorubicin (-7.1 kcal/mol). Overall, the results suggest biflavonoid compounds have the potential to be developed as a chemopreventive agent for cervical cancer.

Keywords: *bioinformatics, molecular docking, amentoflavone, 3,8-Biapigenin, cervical cancer protein.*

INTRODUCTION

One of the cancers that is the leading cause of death in women is cervical cancer. Approximately 240,000 deaths from cervical cancer are confirmed annually (Sung, *et al*., 2021). In Indonesia, cervical cancer cases have been ranked second, with an incidence rate of 0.8% or 98,692 people. Most cases of cervical cancer are caused by infection with HPV16 and 18 (Cohen, *et al*., 2019). One of the strategies to suppress risk factors and treat cervical cancer can take the form of primary treatment, adjuvant therapy, and chemotherapy. While these treatments can increase the survival rate in patients, they can also cause adverse side effects and high toxicity (Maduro, *et al*., 2003). Therefore, there is

Submitted: August 21, 2023
Revised: September 24, 2023
Accepted: September 29, 2023
Published online: October 05, 2023

*Corresponding author: rifki.febriansah@umy.ac.id

a need for alternative chemopreventive agents that have low side effects in cervical cancer patients.

Research on chemopreventive agents for cervical cancer is still being developed, mainly focusing on compounds that exhibit specific targets and high selectivity as protein inhibitors. One such compound with potential as a chemopreventive agent is amentoflavone. Amentoflavone is a polyphenol compound that has been identified in over 120 plants, including *Celaenodendron mexicanum, Cupressus funebris, Garcinia multiflora, Biophytum sensitivum, Rhus succedanea, Hypericum perforatum,* and *Cupressocyparis leylandii*. Amentoflavone is known for anti-inflammatory and anticancer effects (Yu, *et al*., 2017). In addition, Lei, *et al*. discovered that biflavonoid extracts derived from *S. moellendorffii* had a significant inhibitory impact on the growth rates of HCT-116 and HeLa cell lines within the concentration range of 0 μg/mL to 500 μg/mL (Lei, *et al*., 2022). Apart from amentoflavone, another compound showing potential as an anticancer agent is 3.8'-Biapigenin. This compound is commonly found in the *Scutellaria baicalensis* plant. It can be synthesized from various sources, such as flavonoids and bioflavonoids. Biapigenin exhibited anti-cancer effects on HeLa cells while not displaying cytotoxicity towards HaCaT cells. These results suggest that biapigenin could potentially act as a strong agonist for hPPARγ, contributing to its anti-cancer properties (Kim, *et al*., 2011).

In drug discovery, one of the initial steps is *in silico* testing. Target proteins can be identified using the STITCH & STRINGdb bioinformatic method. STITCH is a database that offers data on protein interactions with small molecules, allowing for the assessment of the binding affinity of a chemical compound within a network of interactions (Szklarczyk, *et al*., 2016). On the other hand, STRING is a database that provides data on protein interactions, including physical and functional interactions (Szklarczyk, *et al*., 2019). Computational-based research, such as molecular docking, utilizes computers to design drugs and aims to predict the primary receptor region that binds a ligand (Puspaningtyas, 2013). The molecular docking technique serves as a tool for identifying and optimizing lead compounds, expediting the selection of compounds to be isolated and synthesized during the drug discovery process (Rastini, *et al*., 2019). This study aims to assess the potential of amentoflavone and 3,8'-Biapigenin compounds as protein inhibitors for cervical cancer, employing bioinformatics and molecular docking studies. The findings of this study can serve as a reference for the design and development of new drugs for cervical cancer.

MATERIALS AND METHODS

Prediction of Activity Spectra for Active Substances (PASS) Test

The anticancer activity prediction test with the method is done by accessing the online PASS website via the link (http://www.way2drug.com/passonline/predict.php). Then copy the canonical SMILE obtained on the PubChem server (https://pubchem.ncbi.nlm.nih.gov).

Table 1. Anticancer activity prediction test results with PASS online.

No	Compound	Pa (*Probable activity*) value		
		>0.7	>0.5 - <0.7	<0.5
1.	*Amentoflavone*	-	*Breast cancer, Chemopreventive, Anticarcinogenic.*	***Cervical cancer***
2.	3,8-Biapigenin	-	*Breast cancer, Breast cancer-resistant protein inhibitor, small cell lung cancer.*	***Cervical cancer***

STITCH-STRING Bioinformatic Test

The potential target proteins with compound's molecules search using the STITCH-STRING bioinformatics method. Direct Target Protein was obtained by accessing the STITCH database at https://stitch.embl.de/. In comparison, Indirect Target Protein is done by accessing the website https://string-db.org/. Then the search for cervical cancer proteins was obtained from the NCBI website (https://www.ncbi.nlm.nih.gov/). Furthermore, intersections from the Venn diagram between DTPs - ITPs and total of 1,767 cervical cancer gene databases (Homo sapiens) were obtained through NCBI. The last step, the visualization process using Cytoscape v 3.8.2 application and determined the top 10 protein targets based on the high degree score, which then selected the best two proteins that will be carried out for molecular docking testing.

Receptor Analysis Test with Ramachandran Plot

Target receptor analysis was performed by looking at the PDB profile protein based on Ramachandran Plot. This was done by entering 4 PDB protein codes on the Ramachandran Plot website (https://www.ebi.ac.uk.pdbsum/). This analysis was done to validate the 3D stability of the protein.

Molecular Docking Test

This Molecular Docking test research uses Autodock Vina, Marvin Sketch, Discovery Studio Visualizer, Mgl Tools, and Autodock Tools applications. The target protein was downloaded on the PDB Bank website in PDB format. Then the original ligand preparation was obtained from the target protein file using Autodock Tools. Ligand and protein files were converted using Autodock Tools with PDBQT file format. Then running using Autodock Vina with RMSD <2Å output. The last step is to visualize the docking results using Discovery Studio Visualizer to facilitate researchers in analyzing molecular docking results (Susanti, 2018).

RESULTS

Protein Tracking Using the STITCH-STRING Bioinformatic Test

Before the anticancer activity test using molecular docking, the potential target proteins with compound's molecules was traced using the STITCH-STRING bioinformatics method. Research results obtained using STITCH include data on Direct Target Proteins (DTPs), which are the target proteins that directly interact with amentoflavone or 3,8-biapigenin compounds. On the other hand, the data obtained from STRING consists of Indirect

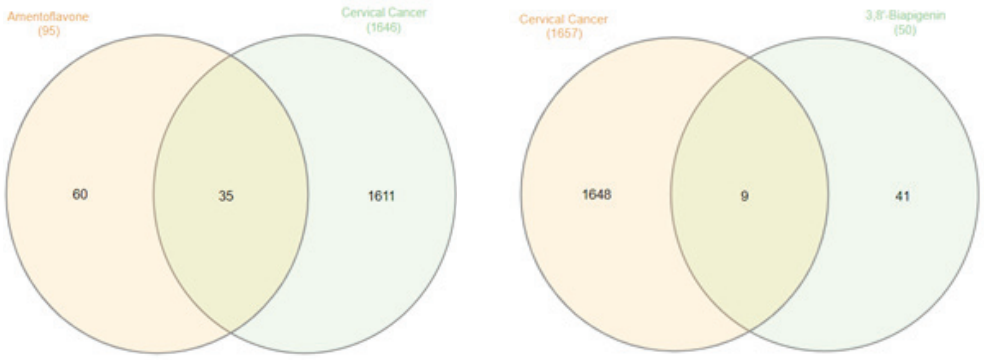

Figure 1. Intersections from the venn diagram between the target proteins of the compound Amentoflavone and target cervical cancer (A) and 3,8-biapigenin with target cervical cancer (B).

Indonesian Journal of Cancer Chemoprevention, June 2023
ISSN: 2088–0197
e-ISSN: 2355-8989

Target Proteins (ITPs), which interact with the DTPs. The results of ITPs data are combined with DTPs data, then potential target proteins with compound's molecules were identified by taking the intersections from the venn diagram between the target proteins of the compound and target cervical cancer. The result as shown in Figure 1.

The results of potential target proteins obtained were visualized with Cytoscape v 3.8.2. The process of selecting protein interaction data is done by topological analysis, a quantitative analysis to determine the critical proteins involved in the disease mechanism parameters used in topology analysis, namely using degree score analysis

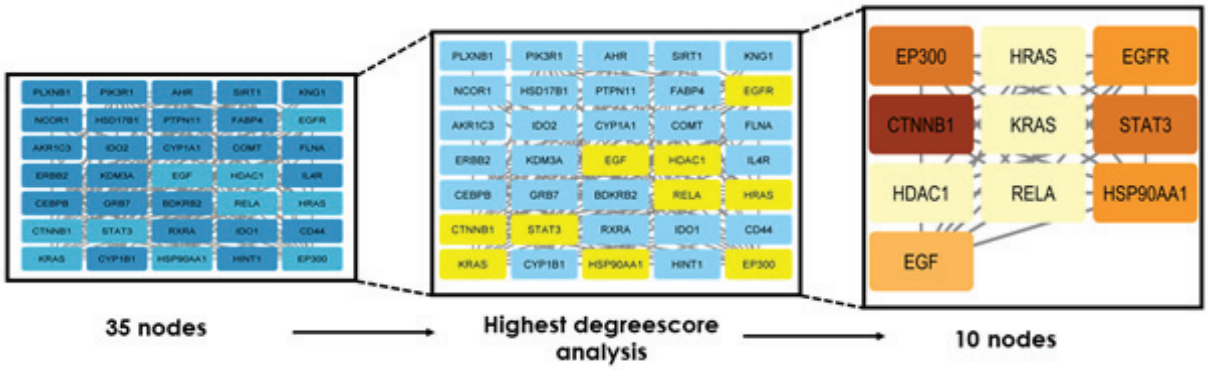

Figure 2. Top 10 highest degree score analysis of potential target proteins amentoflavone.

(Ren, *et al*., 2016). Potential protein targets with the largest Degree Score values are sorted to identify the top 10 proteins. The results showed 35 potential protein targets with amentoflavone, which were then sorted based on the top 10 highest Degree Scores. The results potential target proteins with amentoflavone in Figure 2.

The results of the intersections between the target protein 3,8-Biapigenin and cervical cancer target, as depicted in the Venn diagram, revealed a total of 9 proteins. This limited number of proteins is attributed to the constraints of the 3,8-Biapigenin database. Subsequently, an analysis of the highest degree scores was conducted among these 9 proteins, leading to the identification of the top 5 potential target proteins with the highest degree score values.

Amentoflavone compounds, the types of proteins selected for use as target receptors are EP300 (PDB ID: 7LJE) and STAT3 (PDB ID: 6NJS). Both proteins have the same degree score of 19. In the 3,8-biapigenin compound, the proteins selected as target receptors are CYP1A1 protein (PDB ID: 4I8V) and AKR1C1 protein (PDB ID: 3NTY).

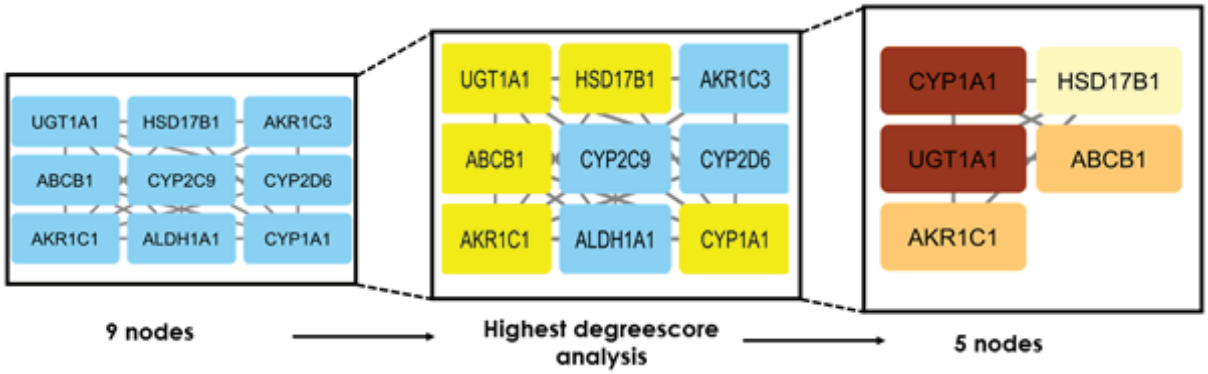

Figure 3. Top 5 highest degree score analysis of potential target protein 3,8-Biapigenin.

Table 2. Top 10 and Top 5 potential target protein of amentoflavone and 3,8-biapigenin compounds.

	Amentoflavon		3 ,8 -Biapigenin	
No	**Protein Symbol**	***Degree Score***	**Protein Symbol**	***Degree Score***
1	CTNNB1	21	UGT1A1	8
2	EP300	19	CYP1A1	8
3	STAT3	19	ABCB1 5	
4	EGFR	18	AKR1C1 5	
5	HSP90AA1	18	CYP2C9 4	
6	EGF	17		
7	HDAC1	15		
8	HRAS	15		
9	KRAS	15		
10	RELA	15		

The two proteins have degree score values of 8 and 5, respectively. The selection of the four proteins is because these types of proteins are found in homo sapiens organisms. Then another factor considered in the section on target proteins is the occurrence of mutations, and in the Protein Data Bank (PDB), the four proteins do not have mutations. Furthermore, the resolution of the four proteins, namely 2.61 Å (EP300), 2.70 Å (STAT3), 2.60 Å (CYP1A1), and 1.87 Å (AKR1C1), is classified as reasonable resolution. All target protein PDB codes selected in this study were obtained from experimental research through the X-Ray Diffraction method.

Protein Analysis Test with Ramachandran Plot

Ramachandran plot analysis is performed to analyze the structural stability of the receptor or target protein. The Ramachandran plot consists of four quadrants or regions, namely most most favourable region (Quadrant I), additional regions (Quadrant II), generously allowed regions (Quadrant III), and disallowed regions (Quadrant IV). The lower the percentage of disallowed areas, the higher the stability and quality of the protein structure because non-glycine amino acid residues in disallowed regions can cause a steric obstruction that can interfere with protein conformation to form stable bonds with ligands or compounds in molecular docking tests (Yuliana, *et al*., 2020). The results of the analysis of the four proteins in this study can be seen in Table 3.

Table 3. Analysis results of four proteins using Ramachandran Plot.

Kode PDB Protein	***Most Favourable Regions***	***Dissalowed Regions***	**Result**
EP300 (7LJE)	94.4%	0.0%	Good & Stable
STAT3 (6NJS)	93.0%	0.0%	Good & Stable
CYP1A1 (4I8V)	88.5%	0.0%	Good & Stable
AKR1C1 (3NTY)	92.2%	0.4%	Good & Stable

Based on the results of protein analysis, it shows that all proteins have stable and good results. This is because the percentage distribution of non-glycine amino acid residues in the most favourable region (Quadrant I) ≥50% and in the dissalowed region (Quadrant IV) ≤ 15% (Yuliana, *et al*., 2020).

Molecular Docking Test

Based on degree score analysis, ramachandran plot and good receptor criteria in the Protein Data Bank, four proteins were selected to be used in this research. The proteins used in

Table 4. Molecular docking results of test compounds against target protein.

No	Compound	Target Protein	RMSD (<2.00 Å)	*Score Docking* (kkal/mol)	Conformation
1.	*Amentoflavone*	EP300	1.823	-8.8	4
		STAT3	1.341	-9.3	2
2.	3,8 -Biapigenin	CYP1A1	1.696	-8.4	8
		AKR1C1	1.569	-8.9	2
3.	Doxorubicin	EP300	1.574	-9.0	6
		STAT3	1.893	-7.1	3
		CYP1A1	1.760	-10.8	2
		AKR1C1	1.882	-8.9	2

this study are EP300 (PDB: 7LJE), STAT3 (PDB: 6NJS), CYP1A1 (PDB: 4I8V), and AKR1C1 (PDB: 3NTY). In addition, the comparison compound used in this research is doxorubicin, a chemotherapy drug employed in the treatment of cervical cancer. Doxorubicin is chosen as the comparison drug because the mechanism of action of doxorubicin is well-studied. It functions by inhibiting DNA synthesis and causing DNA damage in cancer cells, which leads to their death. Understanding its mechanism of action can provide valuable insights when comparing it to other compounds being studied *in silico*. Furthermore, there may be ample data available on the interactions of doxorubicin with cancer cells, including cervical cancer cells. This data can be used to validate and calibrate the in silico models used in the study. The docking score results of test and comparison compounds with protein targets can be seen in Table 4.

Table 5. Visualization of molecular docking.

Compounds	Target Proteins	Interactions	
		Hydrogen Bonds	Hydrophobic Bonds
Amentoflavone	STAT3	TYR A: 640, GLN A: 644, VAL A: 637.	GLU A: 638
Doxorubicin		PRO A: 669	VAL A: 667, ILE A: 659, GLU A: 625, ALA A: 662, ASP A: 661
Amentoflavone	EP300	HIS A:1597, ASN A: 1511.	PRO A: 1439
Doxorubicin		ASP A: 1444, SER A: 1480, LEU A: 1398, HIS A: 1451, GLN A: 1455, PRO A: 1458, LYS A: 1456, TRP A: 1466.	ARG A: 1410, HIS A: 1402.
3,8 -Biapigenin	CYP1A1	ASN A: 245, ASN A: 232.	LEU A : 240, P RO A : 233, ALA A: 234.
Doxorubicin		HIS A: 388, ILE A: 458, CYS A: 457, GLY A: 459, LEU A: 314, SER A: 122, ASP A: 313.	PHE A: 224, GLY A: 316, PHE A: 123, ALA A: 317, LEU A: 496.
3,8 -Biapigenin	AKR1C1	TYR A: 272, LYS A: 270.	ARG A: 276.
Doxorubicin		LYS A : 270, SER A: 271, ARG A: 276, ASN A: 273, ARG A: 223.	TYR A: 24, HIS A: 222, SER A: 221

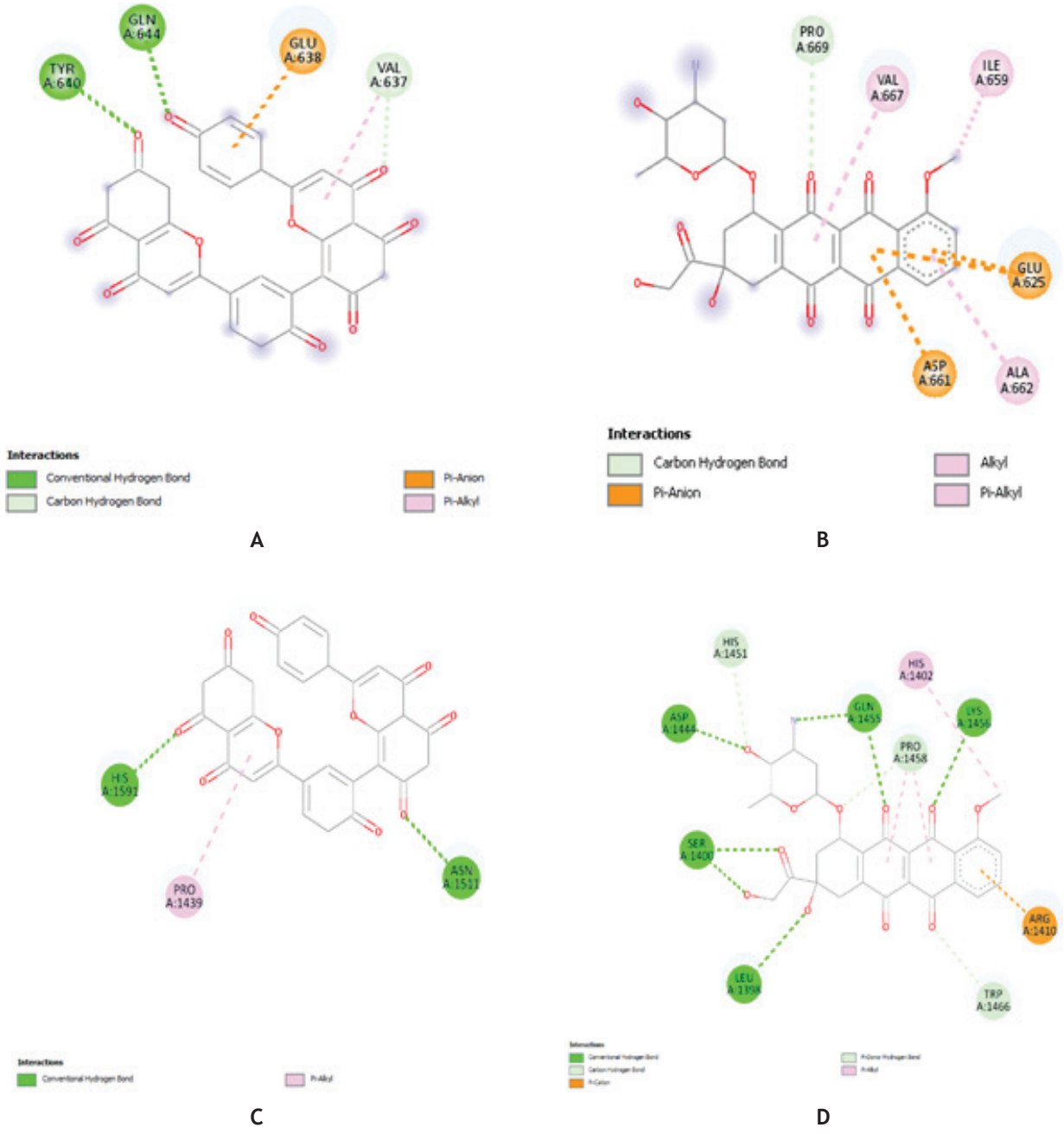

Figure 4. 2D visualization of STAT3 target proteins with (A) amentoflavone, STAT3 target proteins with (B) doxorubicin, EP300 target protein with (C) amentoflavone, and EP300 target protein with (D) doxorubicin.

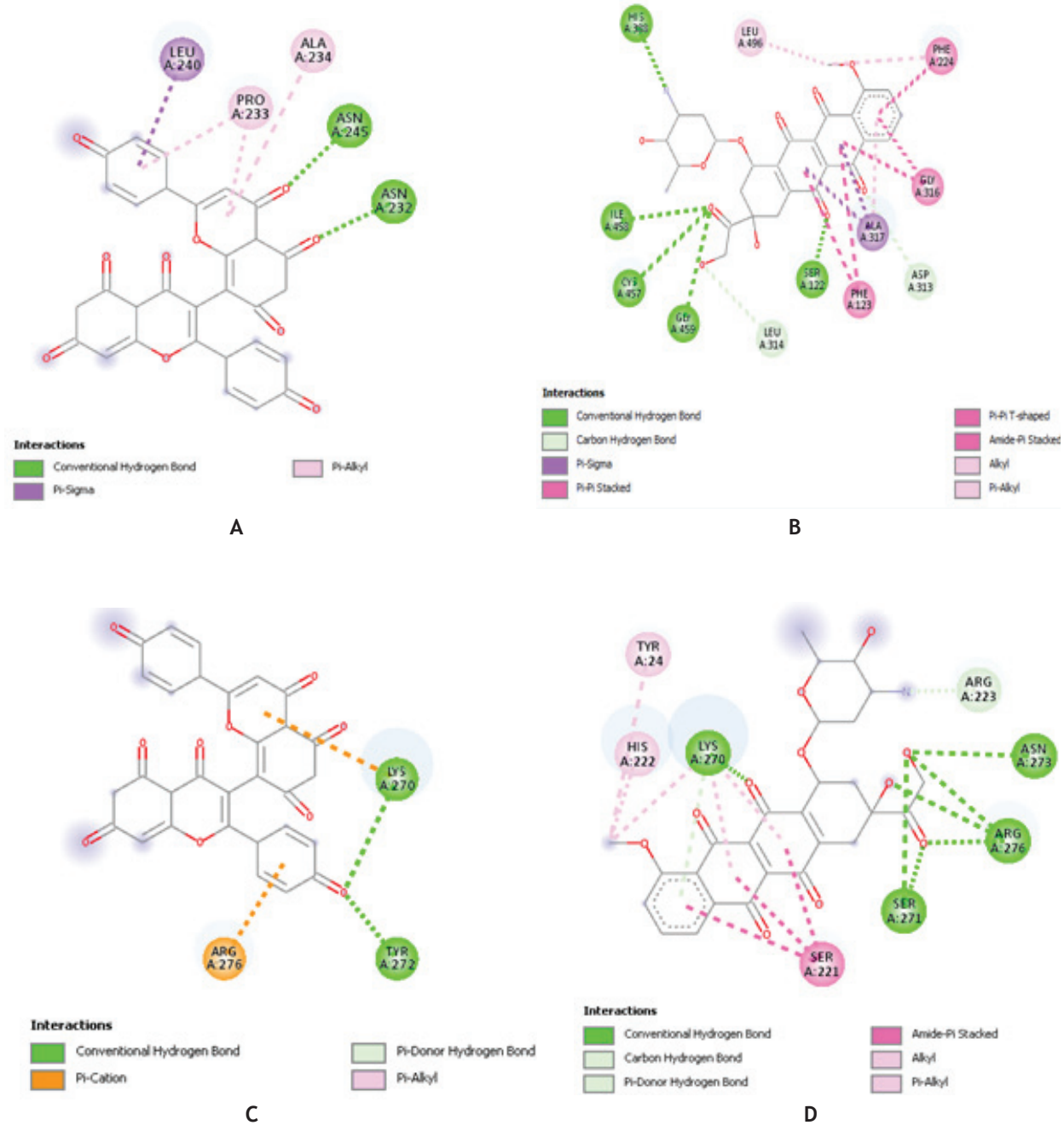

Figure 5. 2D visualization of CYP1A1 target protein with (A) 3,8 -Biapigenin and CYP1A1 target protein with (B) Doxorubicin, AKR1C1 target protein with (C) 3,8 -Biapigenin and AKR1C1 target protein with (D) Doxorubicin.

The best docking score of the test compound was obtained in the interaction of amentoflavone with STAT3 target protein (-9.3 Kcal/mol; RMSD 1.341; conformation 2). The docking score was lower than the comparison compound doxorubicin with STAT3 (-7.1 Kcal/mol; RMSD 1.893; conformation 3). The best docking score of the test compound 3,8-biapigenin was obtained from the interaction of 3,8-biapigenin with AKR1C1 protein (-8.9 kcal/mol; RMSD 1.569; Conformation 2). The binding result showed the same result as the comparator compound doxorubicin (-8.9 kcal/mol; RMSD 1.882; conformation 2). The visualization results can be seen in Table 5.

DISCUSSION

Cervical cancer is one of the significant causes of death. Cervical cancer begins in the cervix, the lower part of the uterus that connects it to the vagina. It typically develops slowly, progressing from cervical cell changes called dysplasia to the formation and spread of cancer cells. Biflavonoid compounds such as amentoflavone and 3,8 biapigenin are thought to have essential roles for anticancer effects. Searching for potential candidates for plant compounds to become drugs can be done by in silico testing. In silico PASS tests were conducted to predict the anticancer potential of amentoflavone and 3,8-biapigenin compounds when tested on a laboratory scale. The basic principle of PASS is based on the relationship between the structure of the compound and its biological activity (Filimonov, *et al*., 2014). The output obtained from the PASS test is the Pa (Probable activity) value, which shows a compound's high biological activity or anticancer activity when tested in the laboratory. The PASS test showed that the amentoflavone and 3,8-biapigenin compounds had Pa values <0.5, 0.331, and 0.345, respectively. This indicates that the cervical anticancer activity of both compounds is expected to be low when tested on a laboratory scale (Chelliah, 2008). This test can be supported by the molecular docking method's prediction of binding affinity.

The STITCH-STRING bioinformatic test was conducted to trace target proteins interacting with amentoflavone and 3,8-biapigenin compounds in the body—parameters used in topology analysis, namely, degree score analysis (Ren, *et al*., 2016). The degree score describes the size of the protein in the interaction network. The larger the size of a protein, the more direct interactions it has (Simos, *et al*., 2015). In addition, the selection of proteins that will be used as target receptors, namely the origin of the organism, protein resolution, and experimental methods used. Based on the results of STITCH-STRING data, the type of protein selected for the target receptor is EP300 with protein code 7JLE and STAT3 with protein code 6NJS with a degree score of 19. At the same time, the 3,8-biapigenin compound protein selected for the target receptor is CYP1A1 protein with protein code 4I8V and AKR1C1 protein with protein code 3NTY with a degree score of 8 and 5, respectively. The selection of the four proteins is because these types of proteins are found in Homo sapiens . The choice of Homo sapiens is based on the drug development in this study, which is expected to be developed in clinical trials in humans and has the same structure as the human body organism. In addition, the resolution value of the protein is less than 3 Å. The lower the resolution value of the receptor, the better the stability of the receptor will be during the molecular docking process (Marcou and Rognan, 2007).

Analysis of receptor stability or target protein structure was carried out using Ramachandran plots. The results of the Ramachandran plot show that the four protein structures have good quality for receptors in the molecular docking test. The Ramachandran diagram represents a polypeptide that systematically varies the phi and psi angles to obtain a stable conformation. The results of the analysis of

non-glycine residues on the entire tested protein showed <15% in the disallowed regions and >80% in the most favourable regions so that the structure of the tested protein was stable and could be used for further tests using molecular docking. This is by research conducted by Ho and Brasseur, 2015 which states that if non-glycine residues are in the disallowed region >15% and the most favourable region <80%, then the protein structure can be said to have poor structural quality (Ho and Brasseur, 2005).

The best results were obtained in binding amentoflavone compounds with STAT3 protein. STAT3 (Signal transducer and activator of transcription 3) protein is an essential factor that acts as a super-regulator in tumors (Yu et al., 2014). STAT3 is a crucial part of Janus Kinase (JAK) and STAT that can mediate cell, apoptosis and cell growth by regulating downstream gene expression (Masjedi, *et al*., 2018). If a compound is to be used as a STAT3 inhibitor, in principle, STAT3 inhibition in tumor treatment is based on targeting the upstream receptors of STAT3 signaling, such as IL-6, EGFR, and JAK tyrosine kinase, or by directly targeting STAT3. The kinase or JAK pathway is a key activator of the STAT3 pathway. As for inhibiting STAT3 directly, it can be done through binding to DNA and domains. In addition, negative STAT3 regulators, such as SOCS (Suppressor of Cytokine Signaling), can also act as direct STAT3 inhibitors (Wang, *et al*., 2022). So, if amentoflavone is to be designed as an anticancer agent by inhibiting STAT3, that is through inhibiting the upstream STAT3 receptor, directly inhibiting STAT3, or inhibiting the negative regulator of STAT3. In addition, STAT3 acts as a multifunctional regulator in the formation, development, and metastasis of cancer. Then the docking score data of compound 3,8-Biapigenin against CYP1A1 protein (-8.4 kcal/mol; RMSD 1.696; Conformation 8) while doxorubicin (-9.0 kcal/mol; RMSD 1.760; conformation 2). These results indicate that the chemotherapeutic agent doxorubicin has a better binding affinity than the test compound 3,8-biapigenin to CYP1A1 protein.

Based on the molecular docking results, the test compound with the best binding affinity is the interaction between Amentoflavone and STAT3 target protein, which has a lower docking score than doxorubicin. Amentoflavone has more hydrogen bonds compared to doxorubicin. Hydrogen bonding is the most robust and stable type of intermolecular bond and is a significant contributor to the stability of the bond between the ligand complex and the target. Therefore, the interaction between ligands and proteins that have more hydrogen bonds will form more potent and more stable interaction bonds (Glowacki, 2013). The bond formed between the comparator compound of the doxorubicin chemotherapy agent only has one hydrogen bond, and more hydrophobic bonds are formed. Hydrophobic bonds are among the weakest bonds but are essential for highly fat-soluble drugs to interact with the fatty layer of membranes or membrane cell walls (Otto and Engberts, 2003). So the interaction between doxorubicin and STAT3 protein is thought to have a weak or less stable bond.

CONCLUSION

Based on the *in silico* test, the compounds amentoflavone and 3,8-biapigenin are believed to exhibit low cervical anticancer activity if tested on a laboratory scale because they have a Pa value <5. According to molecular docking, the strongest bond of the amentoflavone compound, specifically the interaction between amentoflavone and the STAT3 protein, demonstrates a robust and stable bond with a docking score of -9.3 kcal/mol. Amentoflavone's potential for cervical anticancer activity is thought to be greater in inhibiting STAT3 compared to doxorubicin, which has a docking score of -7.1 kcal/mol. In contrast, the best binding of the 3,8-biapigenin compound was observed with the AKR1C1

protein, yielding a docking score of -8.9 kcal/mol. The binding of 3,8-biapigenin with AKR1C1 shares the same docking score as the drug doxorubicin. Therefore, it can be concluded that amentoflavone has a promising potential to be developed as a cervical anticancer agent, targeting the STAT3 protein, while 3,8-biapigenin shows potential for action on the AKR1C1 protein.

ACKNOWLEDGEMENT

The authors would like to thank the Pharmacy Program, Universitas Muhammadiyah Yogyakarta, for their support during the research. The author would also like to thank the Anticancer Research Team for support and funding the research.

REFERENCES

Chelliah, D.A., 2008, Biological Activity Prediction of an Ethno Medicinal Plant Cinnamomum camphora Through Bio-informatics, *Ethnobotanical Leaflets*, **12**, 181–190.

Cohen, P.A., Jhingran, A., Oaknin, A., and Denny, L., 2019, Cervical cancer, *The Lancet*., **393**(10167), 169–182.

Filimonov, D.A., Lagunin, A.A., Gloriozova, T.A., Rudik, A.V., Druzhilovskii, D.S., Pogodin, P.V., and Poroikov, V.V., 2014, Prediction of the biological activity spectra of organic compounds using the pass online web resource, *Chemistry of Heterocyclic Compounds*, **50**(3), 444–457.

Glowacki, E.D., Vladu, M.I., Bauer, S., and Sariciftci, N.S., 2013, Hydrogen-bonds in molecular solids-from biological systems to organic electronics, *J. Mater. Chem. B.*, **31**, 3742-3753.

Ho, B.K., and Brasseur, R., 2005, The Ramachandran plots of glycine and pre-proline, *BMC Structural Biology*, **5**, 1–11.

Kim, J.-K., Shin, S., Lee, J-Y., Lee, S., Lee, E., Jin, Q., *et al.*, 2011, Biapigenin, Candidate of an Agonist of Human Peroxisome Proliferator-Activated Receptor γ with Anticancer Activity, *Bulletin of the Korean Chemical Society*, **32**(8), 2717.

Lei, J., Wang, Y., Li, W., Fu, S., Zhou, J., Lu, D. *et al.*, 2022, Natural green deep eutectic solvents-based eco-friendly and efficient extraction of flavonoids from Selaginella moellendorffii: Process optimization, composition identification and biological activity, *Separation and Purification Technology*, **283**, 120203.

Maduro, J.H., Pras. E., Willemse, P..H.B., and de Vries, E.G.E., 2003, Acute and long-term toxicity following radiotherapy alone or in combination with chemotherapy for locally advanced cervical cancer, *Cancer Treatment Reviews*, **29**(6), 471–488.

Marcou, G., and Rognan, D., 2007, Optimizing fragment and scaffold docking by use of molecular interaction fingerprints, *Journal of Chemical Information and Modeling*, **47**(1), 195–207.

Masjedi, A., Hashemi, V., Hojjat-Farsangi, M., Ghalamfarsa, G., Azizi, G., Yousefi, M., and Jadidi-Niaragh, F., 2018, The significant role of interleukin-6 and its signaling pathway in the immunopathogenesis and treatment of breast cancer, *Biomedicine and Pharmacotherapy*, **108**, 1415–1424.

Otto, S., and Engberts, J.B.F.N., 2003, Hydrophobic interactions and chemical reactivity, *Organic and Biomolecular Chemistry*, **1**, 2809–2820.

Puspaningtyas, A.R., 2013, Docking Molekul Dengan Metoda Molegro Virtual Docker Dari Ekstrak Air Psidium guajava, Linn Dan Citrus sinensis, Peels Sebagai Inhibitor Pada Tirosinase Untuk Pemutih Kulit, *Jurnal Kimia Terapan Indonesia*, **15**(1), 31–39.

Rastini, M.B.O., Giantari, N.K.M., Adnyani, K.D., and Laksmiani, N.P.L., 2019, Molecular Docking Aktivitas Antikanker Dari Kuersetin Terhadap Kanker Payudara Secara *in Silico*, *Jurnal Kimia*,

13(2), 180-184.

Ren, W., Li, Y., Wu, S., Feng, H., and Li, R., 2016, Protein-protein interaction (PPI) network and significant gene analysis of breast cancer, *International Journal of Clinical and Experimental Medicine*, **9**(6), 9033–9043.

Simos, T., Georgopoulou, U., Thyphronitis, G., Koskinas, J., and Papaloukas, C., 2015, Analysis of protein interaction networks for the detection of candidate hepatitis B and C biomarkers, *IEEE Journal of Biomedical and Health Informatics*, **19**(1), 181–189.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F., 2021, Global Cancer Statistics 2020 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, *CA: A Cancer Journal for Clinicians*, **71**(3), 209–249.

Szklarczyk, D., Santos, A., von Mering, C., Jensen, L.J., Bork, P., and Kuhn, M., 2016, STITCH 5 : augmenting protein–chemical interaction networks with tissue and affinity data, *Nucleic Acids Res.*, **44**(D1), 380–384.

Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., *et al.*, 2019, STRING v11 : protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets, *Nucleic Acids Research*, **47**(D1), D607-D613.

Wang, H-Q., Man, Q-W., Huo, F-Y., Gao, X., Lin, H., Li, S-R., *et al.*, 2022, STAT3 pathway in cancers: Past, present, and future, *MedComm*, **3**(2), e124.

Yu, H., Lee, H., Herrmann, A., Buettner, R., and Jove, R., 2014, Revisiting STAT3 signalling in cancer: New and unexpected biological functions, *Nature Reviews Cancer.*, **14**(11), 736–746.

Yu, S., Yan, H., Zhang, L., Shan, M., Chen, P., Ding, A., and Li, S.F.Y., 2017, A review on the phytochemistry, pharmacology, and pharmacokinetics of amentoflavone, a naturally-occurring biflavonoid, *Molecules*, **22**(2), 299.

Yuliana, A., Fitriaji, S.P.H., Mukhaufillah, K.S., and Rizkuloh, L.R., 2020, *In Silico* Study on Testing Antidiabetic Compounds Candidate from Azaphilone Monascus sp., *Microbiology Indonesia*, 14(2), 52–65.