Potential of Kesambi (*Schleichera oleosa*) Active Compound as Antagonist *G-Protein Estrogen Receptor 1* (GPER1) by *In Silico*

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Abstract

Tamoxifen is a treatment for breast cancer patients which can cause side effects of endometrial cancer because it acts as a GPER1 agonist. Active compounds from *Schleichera oleosa* are known to have anticancer potential, such as schleicheol and schleicherastatin, especially their ability to prevent cell proliferation. This research conducted an in silico study to determine the potential of the active compound from *S. Oleosa* as a GPER1 inhibitor. In silico studies include molecular docking and molecular dynamics. The data obtained are binding affinity values, potential energy, RMSD, RMSF, and conformational changes. Active compound candidates with the lowest binding affinity were selected, namely Schleicheol 1 (SCL1), Lupeol (LU), Lupeol acetate (LA), Betulinic acid (BA), and Schleicherastatin 3 (SCR3) with an order of score -8.6, - 8.5, -8.4, -8.4 and -8.4 kcal.mol^{-1}. When complexed with GPER1-Estradiol and GPER1-Tamoxifen, the lowest binding affinity was LU (-8.6 and -8.7 kcal.mol^{-1}). LU binds to the same amino acid as Estradiol and Tamoxifen, namely Leu:271. Based on molecular dynamics, RMSD All (receptor complex) ranged from 3,723 to 5,098 Å, above the normal limit of 3 Å. However, RMSD All shows stability starting from 1.5 ns so that the resulting data can be used. The RMSF value showed higher fluctuations than Tamoxifen at the same binding site as Tamoxifen, including SCL1-T, LU-T, LA-T, and BA-T, which can interfere with the function of the GPER1 receptor. LU, LA, BA, SCL1-T LU-T, and LA-T with GPER1 produce the same structural changes as G15 as GPER1 antagonists. The active compound, especially lupeol, which has the lowest binding affinity, is predicted to have the potential to inhibit GPER1 in silico so that it can be proposed for further testing.

Keywords: Tamoxifen, GPER1, Endometrial Cancer, *Schleichera oleosa*

INTRODUCTION

Tamoxifen is an estrogen receptor antagonist that is very effective in treating breast cancer [1] but can cause serious and significant side effects, such as the possibility of patients developing endometrial cancer [2]. The increased risk of endometrial cancer varies from 1.5 to 6.9 times [3]. The risk of endometrial cancer is not associated with daily doses of tamoxifen but with long-term cumulative use. Three-year endometrial cancer-specific survival decreased significantly from 94% for nonusers to 76% for tamoxifen users over five years [4].

Side effects on endometrial cancer can occur because Tamoxifen acts as an agonist of G protein estrogen receptor 1 (GPER1) in endometrial tissue. GPER1 activation causes EGFR transactivation through metalloproteinase (MMP)-mediated HB-EGF release, activates adenyl cyclase and cAMP accumulation [5], then leads to PKA activation and CREB transcription. Pathways that GPER1 also activates include PI3K [6], calcium mobilization [7], ERK/FAK, and other ion channels. Such activation stimulates the expression of several genes related to cell survival, proliferation, differentiation, migration, and invasion. Therefore, reducing the risk of endometrial cancer for long-term tamoxifen users is an increasingly important cancer prevention target.

One of the plants with pharmacological activity as an anticancer is Kesambi (*Schleichera oleosa*). Kesambi contains active compounds, namely Lupeol, Lupeol acetate Betulin and Betulic acid, which are proven to be anticancer and have a strong inhibitory effect on human endometrial adenocarcinoma [8,9]. Schleicheol 1 and 2, and Schleicherastatin (1-7) are to be a barrier to cancer cell growth [10] by targeting several essential genes such as ERK, PI3K, and Akt which are involved in the development of endometrial cancer, which is included in the GPER1 signaling pathway [11]. Based on these benefits, Kesambi has the potential to be developed in cancer treatment. Therefore, this study aims to determine the potential of active compounds from *S. oleosa* in inhibiting GPER1 *In Silico*.

MATERIAL AND METHODS

Data Mining of Active compound

We selected thirteen Kesambi active compounds (Table 1) with anticancer activity [1,9,10]. Another reason for choosing the active compound is because its physicochemical (Lipinski of Rule) and pharmacokinetic (ADMET)

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properties have been evaluated based on previous research [12]. The active compound is used for virtual screening, which consists of molecular docking and molecular dynamics (Fig. 1). The structure of the active compound was taken from:

1. the KNApSAcK (http://www.knapsackfamily.com/knapsack_core/top.php)
2. PubChem (https://pubchem.ncbi.nlm.nih.gov/)
3. Dr. duke's database (https://phytochem.nal.usda.gov/phytochem/search)

Table 1. Active compound used for the virtual screening

No.	Active Compound	Code
1	Lupeol	LU, CID: 259846
2	Lupeol Acetate	LA, CID: 92157
3	Betulin	BE, CID: 72326
4	Betulinic Acid	BA, CID: 64971
5	Schleicheol 1	SCL1, CID: 10623182
6	Schleicheol 2	SCL2, CID:10837035
7	Schleicherastatin 1	SCR1, CID:10742654
8	Schleicherastatin 2	SCR2, CID:10504189
9	Schleicherastatin 3	SCR3, CID:10765811
10	Schleicherastatin 4	SCR4, CID:11797654
11	Schleicherastatin 5	SCR5, CID: 11797567
12	Schleicherastatin 6	SCR6, CID:10836412
13	Schleicherastatin 7	SCR7, CID:10598763

Because the study aimed to find candidates for GPER1 antagonists, the positive control used G15 (CID: 7433743) as a GPER1 antagonist, and the negative control used Estradiol (CID: 5757) and Tamoxifen (CID: 2733526) as a GPER1 agonist. The 3D chemical structures of the active compounds were obtained via PubChem and downloaded in .sdf format. Before the active compounds can be used for molecular docking, the ligands are converted in the Open Babel software by changing the format (.sdf) to (.pdbqt) [13], then docked using the PyRx software, which functions for virtual screening and docking in AutoDock Vina.

Data Mining of Receptor Target

The membrane receptor G-Protein Estrogen Receptor 1 (GPER1) was modeled using the SWISS MODELL with accession number: Q99527 with the 6LFO protein template from the species Homo sapiens [14,15]. This research was conducted before the alpha fold model was released. So, when protein modeling was carried out, the structure template for the alpha fold model was not yet available.

Molecular Docking of Active Compound with GPER1

The active compound that has been prepared interacts with the GPER1 receptor (Active Compound-GPER1). As a comparison, the active compound was also docked with the GPER1-Estradiol complex (Active Compound-GPER1-Estradiol) and the GPER1-Tamoxifen complex (Active Compound-GPER1-Tamoxifen) to determine their effectiveness when used together with Estradiol and Tamoxifen. Molecular docking is done using an intel core i3-6006U CPU, 2.0GHz, windows 10, and 4GB RAM. AutoDock Vina, included in the PyRx 0.8 package, is used for docking [16]. Autodock Vina was chosen because it adopts pose binding, which is more accurate than Autodock 4, which is in line with the research objective: to support results for wet lab research data with *In Silico* data.

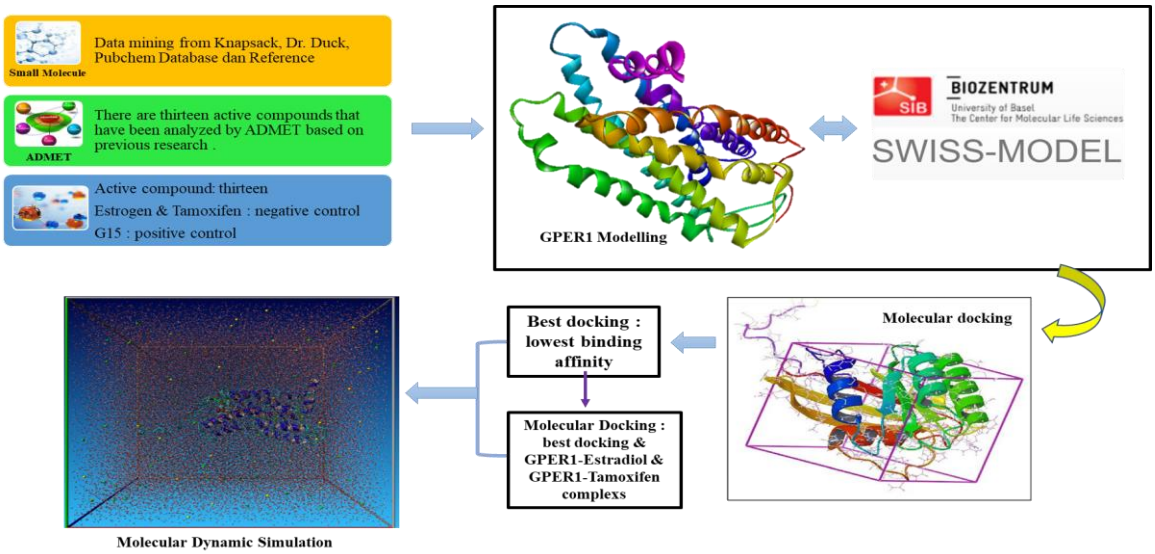

Figure 1. Workflow designed for virtual screening of GPER1 inhibitors

Due to the limited reference regarding the binding pocket on GPER1, all docking operations were carried out using the blind docking method. Blind docking is carried out over the entire receptor surface [17]. Dimensions used: X (49.9543), Y (52.5489), and Z (89.3022), while the center X (131.4273), Y (135.4942), and Z (160.8164). Molecular docking results show a compound with the lowest conformation and binding energy to bind to the target protein. Furthermore, the visualization of the docking results was analyzed using the BIOVIA Discovery Studio to determine their interactions with amino acids [18].

Molecular Dynamic Simulation

The docked receptor and ligand complex targets were selected based on the docking results with the lowest binding. They were subjected to refinement to proceed to molecular dynamics (MD) simulations using YASARA software [19]. The simulation time is set to 4 ns with recording tracks every 25 ps, and the temperature used is adjusted to body temperature, namely 310 K, pH 7.4, pressure: 1, and water density 0.997. The results obtained are potential energy, RMSD, and RMSF.

RESULT AND DISCUSSION

Molecular Docking

Based on molecular docking results, the active compound candidates with the lowest binding affinity values were selected, namely SCL1, LU, LA, BA, and SCR3, with binding affinity values of -8.6, -8.5, -8.4, -8.4, and -8.4 kcal.mol^{-1} respectively. Molecular docking of the active compound with the GPER1-Estradiol complex and the GPER1-Tamoxifen complex was then carried out to compare their interaction capabilities when the kesambi active compound is used together with Tamoxifen. Based on docking results with the GPER1-Estradiol complex, the lowest binding affinity values were LU-E, LA-E, SCL1-E, BA-E, and SCR3-E with values -8.6, -8.4, -8.3, -8.3 and -8.1 kcal.mol^{-1}. Whereas with the GPER1-Tamoxifen SCL1 complex, the lowest binding affinity was LU-T, LA-T, BA-T, and SCR3-T with values -8.6, -8.7, -8.5, -8, 4, and -8.4 kcal.mol^{-1} (Table 2). The lower the energy required, the easier it is for the ligands and proteins to interact.

Based on amino acid interactions, the residue that plays an important role in binding G15 as an antagonist of GPER1 is Arg:208 which forms hydrogen bonds [20]. Hydrogen bond interactions in the system show high stability [21]. Based on the visualization of the results of the tested compounds, SCL1, LU, BA, and SCR3 have the same bond, namely Arg:208, with a van der Waals bond type (Fig. 2). However, this bond is classified as a weak bond [22]. In addition, the binding domain of GPER1 is residue 97-120, 260-280, and 300-302, which play an important role in inhibiting GPER1 [23]. Although the Arg:208 residue of the active compound forms weak bonds, the active compound also forms pi-pi, hydrogen, and hydrophobic bonds with several important residues in the antagonism of GPER1. namely with Trp:104, Tyr267 and Glu:300. This type of bond includes the type of strong bonds. However, molecular dynamic simulations were carried out to find out how the interaction of the test compounds affects GPER1 to support the results of molecular docking and to see whether the interaction causes structural changes similar to those of GPER1 when active or inactive.

Molecular Dynamic Simulation

The stability of the bonding complexes and binding poses obtained in molecular docking is extensively verified by molecular dynamics (MDS) simulations [24]. MD simulation using the YASARA application. Ligands and targets were simulated for 4 ns. The stability of the binding complex was observed every 25 ps recording path, and the values of Potential Energy, Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) were obtained.

Table 2. Molecular Docking Score of 5 Active Compounds with Target Receptors: GPER1.

Phytochemical Target	Binding Affinity (kcal.mol^{-1})		
	Active Compound- GPER1	Active Compound-GPER1-Tamoxifen	Active Compound-GPER1-Estradiol
G15	-9.0	-	-
Estradiol	-7.0	-	-
Tamoxifen	-7.1	-	-
Schleicheol 1	-8.6	-8.6	-8.3
Lupeol	-8.5	-8.7	-8.6
Lupeol Acetate	-8.4	-8.5	-8.4
Betulinic Acid	-8.4	-8.4	-8.3
Schleicherastatin 3	-8.4	-8.4	-8.1

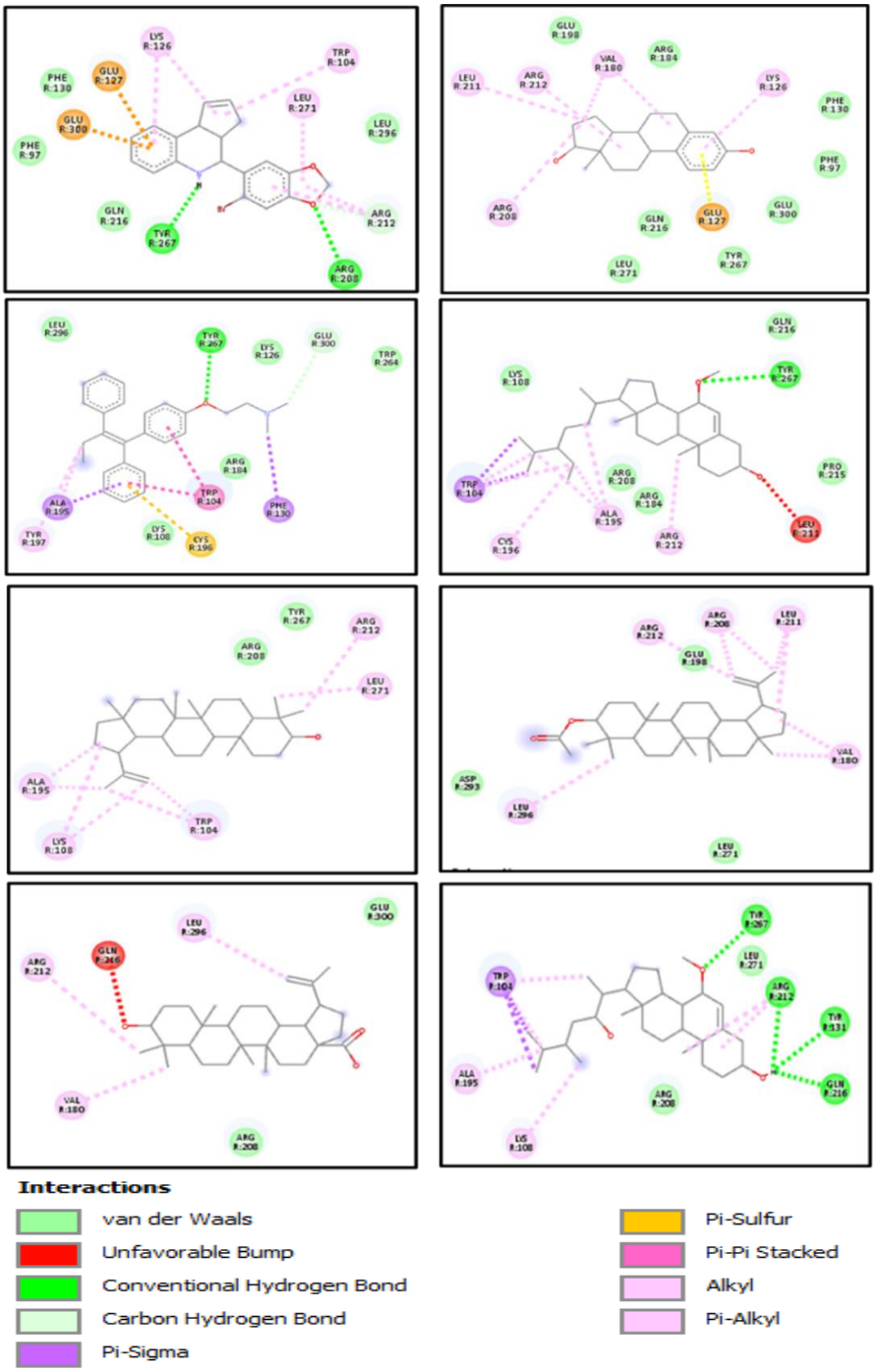

Figure 2. Visualization of molecular docking results, A) G15; B) Estradiol; C) Tamoxifen; D) Schleicheol 1; E) Lupeol; F) Lupeol acetate; G) Betulinic acid; H) Schleicherastatin.

At potential energy, the entire complex shows an equilibrium state, which means that the ligand and protein complexes have reached an equilibrium state until the end of the simulation. Thus, the entire system simulated in this study has been successfully stabilized, marked by a negative potential energy value (Fig. 3A). The lower the system's potential energy, the more stable the molecular geometry in the system [25]. Based on the potential energy results, all active compounds were in a stable state starting at 60 ps with initial values ranging from -1999723.257 to -2612109.446 (kJ.mol^{-1}) (Fig. 3B). RMSD values are used to compare shifts or changes in molecular conformation during the simulation process. The RMSD value used in this analysis is RMSD with receptor complex (RMSD All).

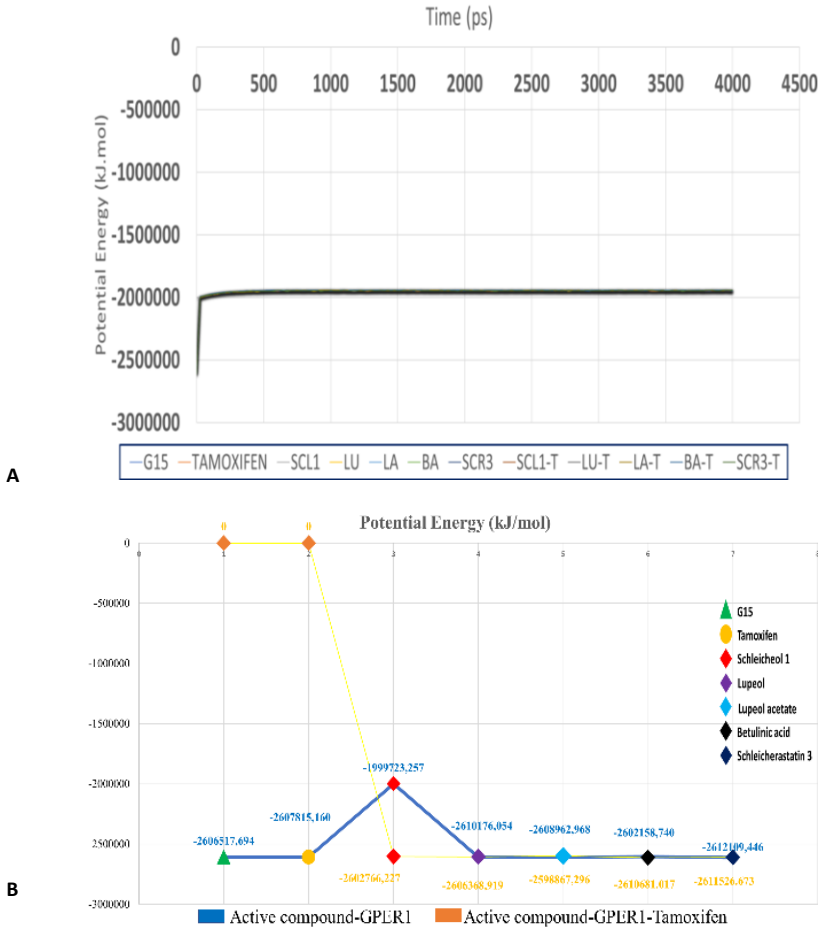

Figure 3. Potential Energy, A) potential energy value for 4 ns; B) initial value of potential energy stability at 60 ps

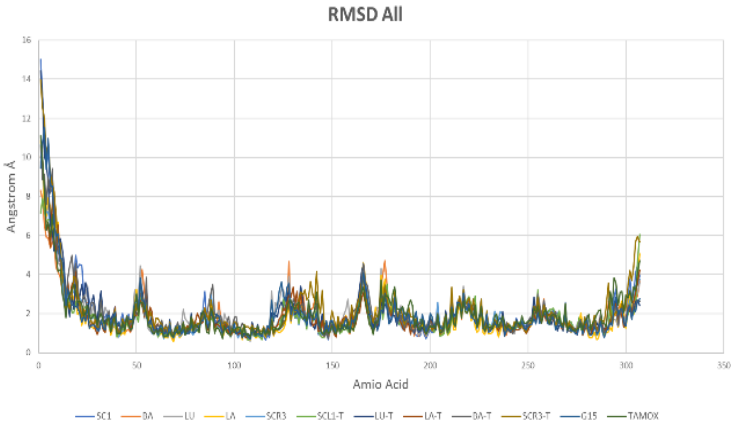

Figure 4. Value of RMSD Active Compound-GPER1 and Active Compound- GPER1-Tamoxifen.

The time required for the entire active compound-GPER1 and the active compound-GPER1-Tamoxifen to reach a fairly stable conformation is relatively the same. The average after the simulation runs at 1.5 ns (1500 ps) until the end of the simulation with an average value RMSD ranges from 3.723 to 5.098 Å (Fig. 4). This value is still relatively high for the RMSD standard, where the maximum is 3 Å for RMSD.

However, a higher RMSD for the receptor complex is not always bad because the RMSD of the active compounds tested shows stability starting at 1.5 ns until the end of the simulation unless it is unstable during the simulation. The high RMSD is likely to undergo a conformational change of the protein in its complex with the ligand.

Co-use of the active compound with tamoxifen significantly reduced the RMSD value at the end of the simulation. However, the SCL1-GPER1-Tamoxifen complex decreased RMSD from an average of 5,098 Å to 3,723 Å. At the same time, the LU-GPER-Tamoxifen also experienced a slight decrease in RMSD value. However, they were not too significant (Fig. 5). Among all the test samples, it was shown that the SCL1-GPER1-Tamoxifen and LU-GPER-Tamoxifen complexes were predicted to have the most stable interactions than the other compounds.

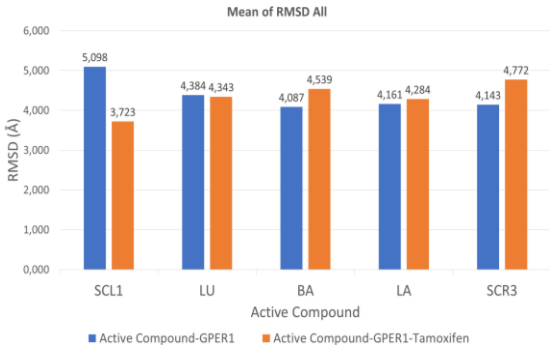

Figure 5. Average Value of RMSD from 1.500 ps to 4.000 ps.

To further verify the stability of the protein-ligand complex, we observed fluctuations in the specific residue where binding occurs. In contrast to RMSD, RMSF parameters were evaluated to determine fluctuations in the interaction of ligands with amino acids during the simulation. RMSF provides specific residual fluctuations, while RMSD provides overall fluctuations. RMSF is calculated for each residue making up the protein, which is to see how much the movement of each residue fluctuates during the simulation.

Based on the RMSF results, there are fluctuations in some amino acid residues of the entire complex (Fig. 6A). RMSF values that are higher than Tamoxifen indicate higher fluctuations and are predicted to disrupt the conformation that has been formed between Tamoxifen and GPER1 so that it loses function in GPER1 activation. SCL1-T, LU-T, and LA-T all have the potential to interfere with Tamoxifen in activating GPER1 (Fig. 6B). The higher RMSF values of Tamoxifen observed in the presence of the added drug/ligand could possibly lead to impaired activation of the previously stable receptor.

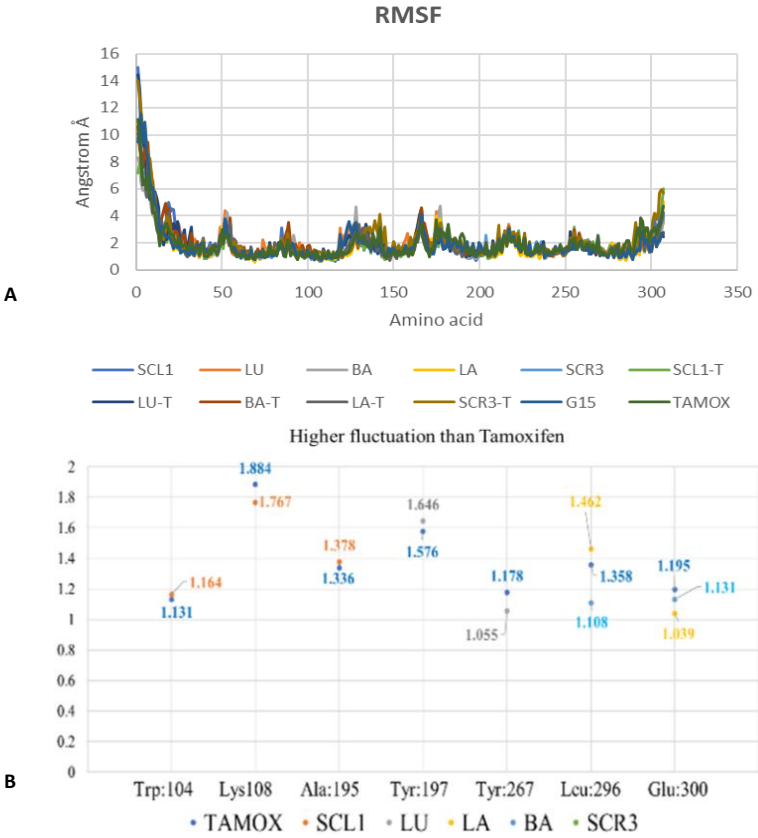

Figure 6. RMSF value and higher fluctuation than Tamoxifen of the active compound with the same amino acid binding site as Tamoxifen, A) RMSF values on all amino acid residues for 4 ns; B) RMSF values at the same amino acid residues as Tamoxifen.

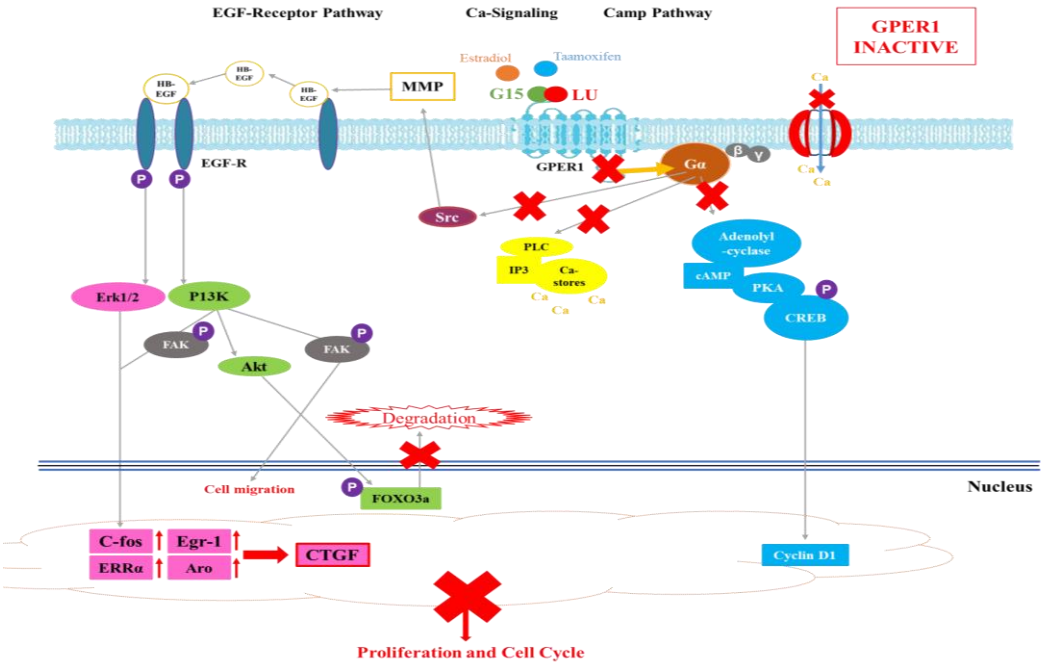

Figure 7. Mechanism when GPER1 is inactive. The active compounds that can prevent GPER1 activation are LU (red) and LA (purple). Inactive GPER1 will not bind to G protein. So, Adenalyl cyclase does not activate, PLC, HBEGF and FOXO3a are not degraded so that the apoptotic function can run.

If we look at the protein structure after molecular dynamic simulation, several active compounds have the same pattern of structural changes as Tamoxifen, namely SCL1, SCR3, BA-T, and SCR3-T, which means that these compound bonds may have the same function as Tamoxifen. Structural changes occur in the 182-196th amino acid residue. The structure changes from a beta sheet connected by a turn to a loop (picture not shown). On the other hand, LU, LA, BA, SCL1-T, LU-T, and LA-T have the same structure as G15. So it is predicted that the binding of the test compound to GPER1 produces the same function as a GPER1 inhibitor, which makes GPER1 inactive and unable to carry out its function.

So, from all the data obtained, the test compound is a potential candidate for further research in preventing Estradiol and Tamoxifen from activating GPER1. In particular is Lupeol because of its lowest binding energy, stable RMSD, and higher fluctuation than tamoxifen. Its binding to GPER1 results in a structure similar to G15, which is predicted to inhibit GPER1 *In Silico*.

The molecular mechanism of Lupeol is predicted to prevent Estradiol and Tamoxifen from activating GPER1. As a result, GPER1 cannot bind to G-proteins so that further pathways in the transcription process, such as activation of adenalyl cyclase, PLC, and Src, cannot take place (Fig. 7). When the activation pathway is prevented, cAMP, CREB and Erk will not be phosphorylated and cannot induce transcription such as cyclin D1 [26] c-fos, Egr-1, ERRα and aromatase in the nucleus so that cell proliferation can be prevented. In addition, PI3-kinase also does not phosphorylate Akt kinase, which phosphorylates the transcription factor FOXO3a in the nucleus [27], so that FOXO3 does not experience degradation and can carry out its function in the apoptotic cell. Therefore, the potential of the active compound as a GPER1 inhibitor can be submitted for further evaluation in the development of cancer treatment related to the side effects of Tamoxifen. The critical thing to note is the selection of drug candidates that do not cause other problems, such as Tamoxifen.

CONCLUSION

Based on the results of molecular docking and molecular dynamics of active compounds from S. oleosa, it can be proposed for further research, especially Lupeol because, based on the data obtained, Lupeol has the lowest binding affinity and its bond with GPER1 produces the same structure as G15 as a GPER1 antagonist so that signaling in the transcription process can be prevented. For further research, it is crucial to study the candidate active compounds so that they do not have a mechanism such as tamoxifen so that treatment development can be obtained without causing other problems.

REFERENCES

[1] Jones, M.E., F.E.V. Leeuwen, W.E. Hoogendoorn. 2012. Endometrial cancer survival after breast cancer in relation to tamoxifen treatment: pooled results from three countries. *Breast Cancer Res*. 14. R91. DOI: 10.1186/bcr3206.

[2] Davies, C., H. Pan, J. Godwin. 2013. Adjuvant Tamoxifen. Longer Against Shorter (ATLAS) Collaborative Group: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet*. 381. 805–816. DOI: 10.1016/S0140-6736(12)61963-1.

[3] Cohen, I. 2004. Endometrial pathologies associated with postmenopausal tamoxifen treatment. *Gynecol. Oncol*. 94. 256–266. Doi: 10.1016/j.ygyno.2004.03.048.

[4] Bergman, L., M.L. Beelen, M.P. Gallee, H. Hollema, J. Benraadt, F.E. van Leeuwen. 2000. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. Assessment of Liver and Endometrial cancer Risk following Tamoxifen. *Lancet*. 9;356(9233). 881-887. DOI: 10.1016/s0140-6736(00)02677-5.

[5] Filardo, E.J., J.A. Quinn, A.R. Frac Kelton, Jr. K.I. Bland. 2002. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol. Endocrinol*. 16. 70–84. [PubMed: 11773440]

[6] Petrie, W.K., M.K. Dennis, C. Hu, D. Dai, J.B. Arterburn, H.O. Smith, H.J. Hathaway, E.R. Prossnitz. 2013. G protein-coupled estrogen receptor-selective ligands modulate endometrial tumor growth. *Obstet*. *Gynecol. Int*. 472720. [PubMed: 24379833].

[7] Tica, A.A., E.C. Dun, O.S. Tica, X. Gao, J.B. Arterburn, G.C. Brailoiu, T.I. Oprea, E. Brailoiu. 2011. G proteincoupled estrogen receptor 1-mediated effects in the rat myometrium. *Am. J. Physiol. Cell Physiol*. 301. C1262–1269. [PubMed: 21865584]

[8] Liu, K., X. Zhang, L. Xie, M. Deng, H. Chen, J. Song, J. Long, X. Li, J. Luo. 2021. Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. *Pharmacol. Res*. 164. 105373. DOI: 10.1016/j.phrs.2020.105373.

[9] Karna, E., L. Szoka, J.A. Palka 2010. Betulinic acid inhibits the expression of hypoxia-inducible factor 1 alpha and vascular endothelial growth factor in human endometrial adenocarcinoma cells. *Mol. Cell Biochem*. 340. 15–20.

[10] Pettit, G.R., N. Atsushi, M.C. Gordon, L.H. Delbert, T. Tamie, I. Chika, R. Roland, M.S. Jean, L.D. Dennis, G. Animesh. 2000. Isolation and structures of Scheicherastins (1-7) and Schleicheols 1 and 2 from the teak forest medicinal tree *Schleichera oleosa*. *J. Nat. Prod*. 63(1). 72-78. DOI: 10.1021/np990346r.

[11] Kaltsas, S., K.N. Syrigos, M.W. Saif. 2014. Pancreatic Cancer in 2014. *JOP. J. Pancreas.* 15(2). 84-86.

[12] Pratiwi, R.I.A., T. Rahayu, N.J. Mubarakati. 2020. *In Silico* screening of *Schleichera oleosa* phytocompounds as estrogen receptors alpha inhibitors for breast cancer. *Journal of Smart Bioprospecting and Technology*. 2(1). 14-21.

[13] O'Boyle, N.M., M. Banck, C.A. James. 2011. Open Babel: An open chemical toolbox. *J Cheminform.* 3. 33. DOI: 10.1186/1758-2946-3-33

[14] Bienert, S., A. Waterhouse, T.A.P. de Beer, G. Tauriello, G. Studer, L.Bordoli, T. Schwede. 2017. The SWISS-MODEL Repository—new features and functionality. *Nucleic Acids Res*. 45(D1). D313–D319.

[15] Waterhouse, A., M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, F.T. Heer, T.A.P. de Beer, C. Rempfer, L. Bordoli, R. Lepore, T. Schwede. 2018. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*. 46(1). W296–W303.

[16] Dallakyan, S., A.J. Olson. 2015. Small-molecule library screening by docking with PyRx. *Methods Mol. Biol*. 1263. 243-50. DOI: 10.1007/978-1-4939-2269-7_19.

[17] Hassan, N.M., A.A. Alhossary, Y. Mu. 2017. Protein-Ligand blind docking using QuickVina-W with inter-process spatio-temporal integration. *Sci. Rep.* 7. 15451. DOI: 10.1038/s41598-017-15571-7.

[18] Dassault Systèmes BIOVIA. 2015. Discovery studio modeling environment, Version 4.5. Dassault Systèmes. San Diego.

[19] Krieger, E., G. Vriend. 2014. YASARA View - molecular graphics for all devices - from smartphones to workstations. *Bioinformatics*. 30(20). 2981-2. DOI: 10.1093/bioinformatics/btu426.
[20] Vidad, A.R., S. Macaspac, H.L. Ng. 2021. Locating ligand binding sites in G-protein coupled receptors using combined information from docking and sequence conservation. *PeerJ*. 9. e12219. DOI: 10.7717/peerj.12219.
[21] Satarker, S., S. Maity, J. Mudgal, M. Nampoothiri. 2022. *In silico* screening of neurokinin receptor antagonists as a therapeutic strategy for neuroinflammation in Alzheimer's disease. *Mol. Divers*. 26(1). 443-466. DOI: 10.1007/s11030-021-10276-6.
[22] Atkins, P., J. de Paula. 2006. Physical chemistry for the life sciences. Oxford University Press. Oxford, UK. 458.
[23] Gutierrez, K.M.P., K.H. Ortega, C.B. Alvarez I.C. Arroyo. 2022. Expression and estrogen regulation of G protein coupled estrogen receptor in human glioblastoma cells. *Oncology Letters*. 24(5). 397.
[24] Anusuya, S., M.M. Gromiha. 2017. Quercetin derivatives as non-nucleoside inhibitors for dengue polymerase: molecular docking, molecular dynamics simulation and binding free energy calculation. *J. Biomol. Struct. Dyn*. 201. 1-24
[25] Fathurrahman, A. 2011. Metode eksperimen. Yrama Widya. Bandung.
[26] Girgert, R., G., Emons, C. Grundker. 2012. Inactivation of GPR30 reduces growth of triple-negative breast cancer cells: possible application in targeted therapy. *Breast Cancer Res. Tre*at. 134. 199–205. DOI: 10.1007/s10549-012-1968-x.
[27] Taylor, A.H., V. Kalathy, M. Habiba. 2014. Estradiol and tamoxifen enhance invasion of endometrial stromal cells in a three-dimensional coculture model of adenomyosis. *Fertil. Steril*. 101. 288–293. DOI: 10.1016/j.fertnstert.2013.09.042.