Pharmacy Reports

https://doi.org/10.51511/pr.77

RESEARCH ARTICLE

Open Access

Molecular docking of several compounds in *katuk* (*Sauropus androgynus* L.) leaves as antibreast cancer in AKT1 protein

Ni Nyoman Ota Sutjiningsih, Azzaima Ayu Ulisya, Amalda Utami, Christine Natalia, Fakhira Chairunnisa Mumtaz, Nola Rohmi Eka Yulanda, Victoria Rekina Sari, Winni Nur Auli, Anjar Hermadi Saputro*

Departement of Phramacy, Faculty of Science, Institut Teknologi Sumatera, Indonesia

*Corresponding author: Jl. Terusan Ryacudu, Way Huwi, Jati Agung, South Lampung 35365, Lampung, Indonesia.
Email: anjar.saputro@fa.itera.ac.id

Abstract: Breast cancer represents a leading cause of cancer-related mortality among women worldwide. *Katuk* leaves (*Sauropus androgynus* L.) demonstrate potential as anticancer agents through their ability to inhibit metastatic processes. The AKT1 protein plays a critical role in preventing apoptosis in breast cancer cells, making it an important therapeutic target. This study employed molecular docking analysis to evaluate the binding affinity of bioactive compounds from *katuk* leaves to the AKT1 protein. The docking methodology involved protein preparation using structures obtained from the Protein Data Bank (PDB ID 3O96), followed by ligand preparation and validation using AutoDockTools version 1.5.6. Chemical interaction analysis was performed using BIOVIA Discovery Studio 2021 software. The binding energy analysis encompassed one native ligand, one reference drug (afuresertib), and five *katuk*-derived compounds: kaempferol, catechin, coumarin, squalene, and phytol. The respective binding energies were determined as -12.59, -8.70, -5.92, -6.44, -5.05, -8.11, and -6.70 kcal/mol. Among the tested compounds, squalene exhibited the strongest binding affinity (-8.11 kcal/mol), demonstrating superior interaction with the AKT1 protein compared to other *katuk*-derived bioactive compounds. The in silico screening results indicate that bioactive constituents in *katuk* leaves possess favorable binding characteristics for breast cancer protein targets, with squalene showing particular promise as a natural AKT1 inhibitor.

Keywords: AKT1, breast cancer, molecular docking, *Sauropus androgynus*

Introduction

Health is a valuable asset that supports human life and well-being. A healthy body enables individuals to remain active without hindrance, while good health ensures stable mental and emotional well-being. Recently, numerous diseases have emerged and become significant public health challenges. Among the most life-threatening of these diseases is breast cancer, which is widely recognized as a deadly malignancy, particularly affecting women. According to the World Health Organization (WHO), there were more than 2.3 million new cases of breast cancer worldwide in 2022, resulting in 670,000 deaths globally. Breast cancer remains the leading cause of cancer-related deaths among women [1]. Current treatment options for breast cancer include surgery, radiotherapy, chemotherapy, and targeted therapy. Although various cancer therapy methods have been developed, breast cancer frequently demonstrates treatment resistance and causes significant side effects. Therefore, ongoing research is essential to identify new therapeutic agents that are more selective, effective, and associated with minimal adverse effects.

Katuk leaves (*Sauropus androgynus* L.) represent a plant species that has been used medicinally for centuries in Southeast Asia due to its numerous health benefits. *Katuk* leaves contain various bioactive components, including essential nutrients, vitamins, minerals, and antioxidants [2]. Active compounds present in *katuk* leaves, such as flavonoids and polyphenols, demonstrate potential to inhibit cancer cell growth and metastasis [3]. Additionally, compounds such as kaempferol, which exhibit structural similarities to estrogen, may play a protective role against breast cancer development [4]. Several studies have investigated the therapeutic potential of *katuk* leaves in breast cancer treatment using both in silico and in vitro approaches.

The AKT1 protein represents one of the primary targets in breast cancer research due to its essential role in various cellular signaling pathways that regulate cell growth, proliferation, and survival. AKT1, also known as Protein Kinase B (PKB), is a critical component of the PI3K/AKT/mTOR pathway that frequently undergoes abnormal activation in various cancer types, including breast cancer [5]. AKT1 activation can lead to uncontrolled cell growth and apoptosis inhibition, thereby contributing to cancer progression and therapy resistance. Consequently, AKT1 inhibition using specific compounds represents a promising strategy for developing more effective and selective cancer therapies.

Molecular docking is a computational method used to predict interactions between ligands and target proteins. This approach proves particularly valuable in early drug discovery phases, as it enables researchers to evaluate the potential of compounds as inhibitors or modulators of target protein activity. While *katuk* leaves have been studied for their anticancer potential, a critical knowledge gap remains regarding how their bioactive compounds, such as flavonoids and polyphenols, specifically interact with the AKT1 protein—a key driver of breast cancer progression and therapy resistance. Previous research has not systematically explored the structural basis of these compounds' binding affinity or their capacity to inhibit AKT1 activity, nor has it established connections between computational predictions and experimental validation. In this study, molecular docking was employed to assess the binding affinity and interaction patterns of *katuk*-derived compounds with AKT1, enabling systematic screening for potential inhibitors. This in silico approach provides a crucial foundational step toward identifying novel AKT1-targeted therapies, addressing the unmet clinical need for selective, low-toxicity treatments in breast cancer management, and establishing connections between computational predictions and actionable therapeutic strategies.

Method

Tools and materials

The computational analysis was performed using standard computer hardware. Software applications employed for this computational study included AutodockTools with MGLTools 1.5.6, BIOVIA Discovery Studio 2021 Client, the RCSB PDB database, PubChem, and SwissADME. The three-dimensional macromolecular structure of AKT1 was obtained from the RCSB Protein Data Bank (www.rcsb.org) using PDB ID 3O96.

Protein preparation

The protein preparation process involved obtaining the macromolecular structure containing the target protein and its associated ligands for subsequent research applications. The macromolecular structure was retrieved from the Protein Data Bank website (http://www.rcsb.org/pdb/). This study utilized PDB code 3O96, which corresponds to the AKT1 protein structure. The structure was downloaded in .pdb format for further processing. AutodockTools software was employed during the protein preparation phase. The preparation process included chain separation of the AKT1 protein, removal of the native ligand and associated residues, and structural optimization of the selected chains through water molecule removal and polar hydrogen atom addition. The final protein and ligand structures were saved in .pdbqt format for subsequent docking simulation [6].

Validation process

Method validation was conducted by positioning the Afuresertib ligand within the AKT1 protein binding site using AutoDockTools version 1.5.6. The validation assessment employed the Root Mean Square Deviation (RMSD) parameter, with an acceptable RMSD value defined as ≤ 2 Å to satisfy validation criteria. Methods meeting this RMSD threshold were considered valid and suitable for proceeding with the molecular docking analysis [7].

Ligand preparation

The selected ligands comprised phenolic compounds derived from *Sauropus androgynus* (L.) Merr., specifically kaempferol, squalene, catechin, coumarin, and phytol. Three-dimensional structures of these ligands were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and subsequently converted from .sdf to .pdb format using the SwissADME platform [8].

Molecular docking

The molecular docking analysis involved docking five ligands (kaempferol, squalene, catechin, coumarin, and phytol) to the AKT1 protein using AutoDockTools

software version 1.5.6. Both protein and ligand files were prepared in .pdbqt format and imported into AutoDockTools. The grid box dimensions were established at 21 × 43 × 34 units, positioned at coordinates X = 9.657, Y = -7.762, Z = 10.604, matching the validation grid box specifications. The docking process involved configuring grid parameters through the Grid menu, followed by Output and Save GPF commands. Docking parameters were established by accessing the Macromolecule menu to set rigid filename and receptor selection. Ligand parameters were configured through the Docking menu by selecting the appropriate ligand file in .pdbqt format. Energy parameters were adjusted via the Search Parameter menu using Genetic Algorithm settings with specified GA runs. Results were saved in .dpf file format before executing the docking simulation. The completed process generated output files in .dlg format, and docking results were subsequently compared with reference compounds [8].

Visualization of molecular docking

Visualization and analysis of ligand-protein interactions were performed using BIOVIA Discovery Studio 2021 software. Docking complexes generated by AutoDockTools version 1.5.6 were imported into BIOVIA Discovery Studio 2021 for detailed interaction analysis and structural visualization [8].

Results

The binding energy of the native ligand to the AKT1 protein was -12.59 kcal/mol. The binding energies of the test compounds kaempferol, catechin, coumarin, squalene, and phytol were -5.92, -6.44, -5.05, -8.11, and -6.70 kcal/mol, respectively (Table 1). Among the test compounds, squalene exhibited the lowest binding energy at -8.11 kcal/mol, while coumarin demonstrated the highest binding energy at -5.05 kcal/mol. Lower binding energy values indicate that less energy is required to form bonds or interactions between a ligand and its receptor.

Two-dimensional visualization analysis was performed to examine protein-ligand interactions for both native and candidate ligands (Figure 1). The native ligand IQO demonstrated eight distinct interactions, including conventional hydrogen bonds, carbon-hydrogen bonds, unfavorable donor interactions, Pi-cation bonds, Pi-sigma bonds, stacked Pi-Pi interactions, alkyl bonds, and Pi-alkyl bonds. Conventional hydrogen bonds involved the amino acid residue Asn54, while carbon-hydrogen bonds engaged Tyr272. Unfavorable donor interactions occurred with Ser206 and Lys265. Pi-cation interactions involved Arg273, Pi-sigma bonds engaged Leu210, and stacked Pi-Pi interactions occurred with Trp80. Alkyl bonds formed with Leu64, and Pi-alkyl bonds involved Val270.

Table 1. Binding energy values of *katuk* leaf ligands to AKT1

Ligands	Binding energy (kcal/mol)
Native ligand	-12.59
Kaempferol	-5.92
Catechin	-6.44
Coumarin	-5.05
Squalene	-8.11
Phytol	-6.70
Afuresertib	-8.70

Afuresertib exhibited nine interactions with the AKT1 protein, encompassing carbon-hydrogen bonds, Pi-anion interactions, Pi-carbon donor hydrogen bonds, Pi-sigma bonds, stacked Pi-Pi interactions, amide-Pi stacked bonds, alkyl bonds, and Pi-alkyl bonds. Conventional hydrogen bonding involved amino acid residues Asp292, Thr82, and Trp83. Carbon donor hydrogen bonds engaged Asn53 and Gln79, while Pi-sigma bonds involved Val270. Alkyl and Pi-alkyl bonds were formed with Lys268 and Leu210.

Kaempferol visualization revealed four distinct chemical interactions: conventional hydrogen donor bonds, Pi-sigma bonds, stacked Pi-Pi interactions, and Pi-alkyl bonds. The amino acid residues involved in these interactions included Ala58, Trp80, and Val270.

Two-dimensional visualization of catechin binding to the AKT1 receptor showed conventional hydrogen donor bonds and Pi-alkyl interactions. Conventional hydrogen donor bonds involved amino acid residues Thr81, Asp292, and Gln79, while Pi-alkyl bonds engaged Ile84 and Arg273.

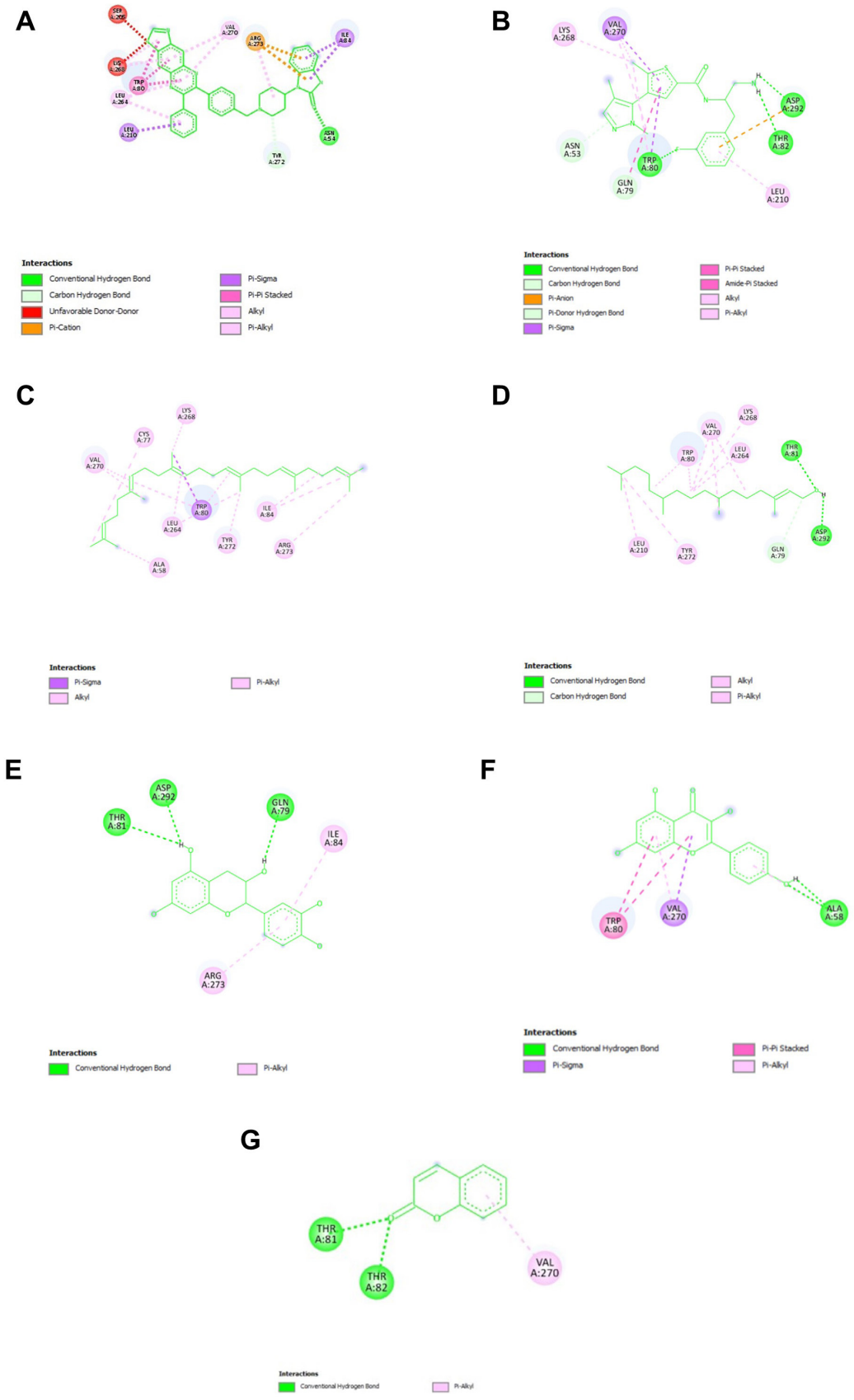

Figure 1. Two-dimensional molecular interaction diagrams showing binding modes of various ligands to the AKT1 protein active site: A) native ligand, B) afuresertib, C) kaempferol, D) catechin, E) squalene, F) phytol, and G) coumarin. Diagrams illustrate key protein-ligand interactions including hydrogen bonds, hydrophobic contacts, and electrostatic interactions.

Squalene visualization demonstrated three types of molecular interactions: Pi-sigma bonds, alkyl bonds, and Pi-alkyl bonds. The Pi-sigma interaction involved Trp80, while alkyl and Pi-alkyl bonds engaged eight amino acid residues: Val270, Cys77, Lys268, Leu264, Ala58, Tyr272, Ile84, and Arg273.

Phytol visualization identified interactions with amino acid residues including Leu210, Tyr272, Gln79, Asp292, Thr81, Trp80, Val270, Leu264, and Lys268. Coumarin visualization revealed two types of interactions: conventional hydrogen bonds and Pi-alkyl bonds. Conventional hydrogen bonds involved Thr81 and Thr82, while Pi-alkyl bonds engaged Val270. Among all seven compounds analyzed, squalene produced the most extensive residue interactions, with eight residue interactions through alkyl and Pi-alkyl bonds.

Discussion

Molecular docking is a computational method that predicts binding affinity between ligands and receptor proteins. This technique has evolved into a important tool in pharmaceutical development [9]. This research included method validation and molecular docking analysis of the target protein. Method validation represented the initial stage, involving docking of the AKT1 target protein with its native ligand from PDB ID 3O96. Subsequently, molecular docking of kaempferol, catechin, coumarin, squalene, and phytol compounds against the AKT1 protein was conducted.

AKT1 protein validation results demonstrated that the structural model accurately represented the native protein. Crystallographic structure refinement of AKT1 using Avogadro software, which included hydrogen atom addition and appropriate protonation state assignment, ensured that model conditions approximated the physiological state. Homology validation confirmed sequence similarity with the native protein. The re-docking methodology using AutoDock resulted in accurate ligand position prediction, and the implementation of positive and negative control compounds further validated the docking approach, demonstrating AutoDock's capability to distinguish between binding and non-binding ligands.

Analysis of docking results using BIOVIA software identified key interactions, including hydrogen bonding and hydrophobic interactions, between active compounds in *katuk* leaves and the AKT1 active site. Low binding energy values indicated high affinity, supporting the compounds' potential as AKT1 inhibitors. These results demonstrated consistency with existing experimental data, confirming the validity of the computational predictions.

Active compounds in *katuk* leaves, including kaempferol, catechin, coumarin, squalene, and phytol, were identified through literature review as potential anti-breast cancer agents. To evaluate their efficacy, molecular docking simulations were performed using AutoDock Tools to assess their binding affinities to the AKT1 protein, a recognized therapeutic target in breast cancer treatment. Binding energy values, which reflect the stability of ligand-receptor interactions, were determined by replacing the native ligand of AKT1 with each test compound. Lower (more negative) binding energy values indicate stronger interactions, as they correlate with reduced free energy required to form stable ligand-protein complexes. This approach enables identification of compounds with promising inhibitory potential against AKT1.

Among the tested ligands, squalene exhibited the highest binding affinity (-8.11 kcal/mol) compared to other *katuk*-derived compounds, suggesting superior potential as an anti-breast cancer agent. Notably, afuresertib, a reference AKT1 inhibitor, demonstrated slightly stronger binding energy (-8.70 kcal/mol), serving as a benchmark for comparison. The results highlighted squalene's competitive binding efficiency, positioning it as a viable natural alternative for targeting AKT1. These findings underscore the significance of molecular docking in predicting ligand-receptor interactions and support further investigation into squalene's therapeutic applications in breast cancer treatment.

The native ligand IQO exhibited eight distinct interaction types with AKT1, including conventional hydrogen bonds with Asn54, hydrocarbon bonds with Tyr272, and pi-cation interactions with Arg273. Notably, residues such as Ser206 and Lys265 contributed to unfavorable donor interactions, while alkyl and pi-alkyl bonds involved Leu64 and Val270, respectively. These diverse interactions likely underpin IQO's high binding affinity (-12.59 kcal/mol), reflecting its structural compatibility with AKT1. Similarly, the reference inhibitor afuresertib demonstrated nine interaction types, including carbon-hydrogen bonds with Asp292, Thr82, and Trp83, pi-anion interactions, and alkyl bonds with Lys268 and Leu210. The extensive network of interactions, particularly involving residues critical for AKT1's catalytic activity, aligned with its

strong binding energy (-8.70 kcal/mol), positioning it as a robust synthetic benchmark.

Among the *katuk*-derived ligands, squalene displayed the most extensive interaction profile, forming bonds with eight residues through pi-sigma (Trp80), alkyl, and pi-alkyl interactions. This broad residue engagement correlated with its superior binding energy (-8.11 kcal/mol) relative to other natural compounds, suggesting enhanced stability within the AKT1 binding pocket. In contrast, kaempferol and catechin exhibited fewer interactions, such as conventional hydrogen bonds with Ala58/Trp80 and Thr81/Asp292, respectively, consistent with their weaker binding affinities (-5.92 and -6.44 kcal/mol). Phytol and coumarin showed intermediate residue interactions, while coumarin displayed only two bond types, further corroborating its low binding energy (-5.05 kcal/mol). The correlation between interaction multiplicity and binding energy underscores the significance of residue engagement in stabilizing ligand-receptor complexes. These findings highlight squalene's potential as a natural AKT1 inhibitor, with its interaction profile rivaling synthetic counterparts, warranting further exploration of its therapeutic efficacy against breast cancer.

Conclusion

The molecular docking analysis revealed that squalene, a natural compound from *katuk* leaves, exhibited the highest binding affinity (-8.11 kcal/mol) to AKT1 among the tested phytochemicals, driven by its extensive interaction profile involving eight residues through pi-sigma, alkyl, and pi-alkyl bonds. While slightly weaker than the synthetic inhibitor afuresertib (-8.70 kcal/mol), squalene's binding efficiency surpassed other *katuk*-derived ligands, such as kaempferol (-5.92 kcal/mol) and coumarin (-5.05 kcal/mol), which demonstrated fewer interactions and lower stability. These findings position squalene as a promising natural AKT1 inhibitor, warranting further experimental validation to explore its therapeutic potential in breast cancer treatment.

Acknowledgments

The authors acknowledge Institut Teknologi Sumatera for the support provided during this research.

Funding

This research received no external funding.

Declaration of interest

The authors declare no conflicts of interest with any private, public, or academic parties related to the information contained in this manuscript.

Author contributions

Conceptualization: AHS, NNOS, WNA; Methodology: AAU, AU, CN, FCM, NREY, VRS, WNA; Investigation: WNA, AHS; Writing – Original Draft: NNOS, AAU, AU, CN, FCM, NREY, VRS, AHS; Writing – Review & Editing: AHS.

Received: August 27, 2024

Revised: April 30, 2025

Accepted: April 30, 2025

Published: May 13, 2025

References

1. Zhang Y, Ji Y, Liu S, Li J, Wu J, Jin Q, et al. Global burden of female breast cancer: new estimates in 2022, temporal trend and future projections up to 2050 based on the latest release from GLOBOCAN. Journal of the National Cancer Center. 2025. https://doi.org/10.1016/j.jncc.2025.02.002
2. Nurdianti RR, Nuryana RS, Handoko A, Hernaman I, Ramdani D, Jayanegara A, et al. Nutritional compositions of Katuk leaves and their supplementation to hays of different quality: an in vitro study. J Agric Sci. 2023;161: 428-437. https://doi.org/10.1017/S002185962300031X
3. Hazafa A, Rehman KU, Jahan N, Jabeen Z. The Role of Polyphenol (Flavonoids) Compounds in the Treatment of Cancer Cells. Nutr Cancer. 2020;72: 386-397. https://doi.org/10.1080/01635581.2019.1637006
4. Wang X, Yang Y, An Y, Fang G. The mechanism of anticancer action and potential clinical use of kaempferol in the treatment of breast cancer. Biomedicine & Pharmacotherapy. 2019;117: 109086. https://doi.org/10.1016/j.biopha.2019.109086
5. Chu N, Salguero AL, Liu AZ, Chen Z, Dempsey DR, Ficarro SB, et al. Akt Kinase Activation Mechanisms Revealed Using Protein Semisynthesis. Cell. 2018;174: 897-907.e14. https://doi.org/10.1016/j.cell.2018.07.003
6. Ponnulakshmi R. Molecular docking analysis of Bcl-2 with phytocompounds. Bioinformation. 2020;16: 468-473. https://doi.org/10.6026/97320630016468
7. López-Camacho E, García-Godoy MJ, García-Nieto J, Nebro AJ, Aldana-Montes JF. A new multi-objective approach for molecular docking based on rmsd and binding energy. Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics). 2016;9702: 65-77. https://doi.org/10.1007/978-3-319-38827-4_6

8. Prasetiawati R, Permana B, Soni D, Agung SN. Studi penambatan molekul senyawa turunan xanton dari kulit buah manggis (Garcinia mangostana L.) terhadap ER-α (reseptor estrogen alfa) dan ER-β (reseptor estrogen beta) sebagai antikanker payudara. Jurnal Ilmiah Farmako Bahari. 2019;9: 45-52. https://doi.org/10.52434/jfb.v9i1.642

9. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023;13. https://doi.org/10.1038/s41598-023-40160-2