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Research Article

Molecular Mechanism of Capsaicin from (*Capsicum Annuum* L.) on Expression of MAPK1 And AKT1 Protein as Candidate of Anticancer Drugs: *In Silico* Study

Mohammad Sukmanadi[1,*], Sri Agus Sudjarwo[2], Mustofa Helmi Effendi[3]

ABSTRACT

One of the most important compounds in *Capsicum annuum* L. is capsaicin, capsaicin is a secondary metabolite of the *Capsicum Annuum* L. plant. In the pharmaceutical field in addition to relieving pain or pain, capsaicin is also known to have anticancer activity because it inhibits certain oncogenic proteins. Further screening of the capsaicin compound against the oncogenic protein produced in the HCC pathogenesis signaling is needed. Screening components in *Capsicum annuum* L. against MAPK1 and AKT1 target proteins is the initial stage of drug discovery. MAPK1 and AKT1 protein bundles and capsaicin ligand bundles that were prepared previously in Autodock 4.0 were molecular dockings (molecular docking). After molecular docking, it was found that capsaicin binds to MAPK1 / ERK with the free energy of Gibbs of -5.5 Kcal/mol and AKT1 of -6.7 Kcal/mol. The free energy of Gibbs is so negative that it is ensured that the reaction will take place spontaneously and lead to high affinity. The data that has been obtained, capsaicin in *Capsicum annuum* L. has a high affinity for MAPK1 and AKT1 receptor/protein targets with the binding energy of -5.5 Kcal/mol and -6.7 Kcal/mol and Potential Activity Score (Pa) equal to 0,690 for preneoplastic treatment, 0.590 for apoptosis agonist, and 0.366 for antineoplastic activity and accordingly become candidates for anticancer drugs.

Key words: AKT1, Anticancer, Capsaicin, *Capsicum annuum* L., MAPK1.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant tumor that attacks the liver. The prevalence of malignant tumors is very high, ranked fourth globally as malignant cancer with an incidence rate of 5.3% compared to other cancers.[1] Pathophysiological HCC arises because of chronic exposure to hepatocytes that can cause epigenetic and genetic changes that lead to the induction of oncogenic proteins and/or activation of tumor suppressor genes. This pathogenesis occurs in four important signaling pathways (WNT, TGFß, PI3K/Akt, and RAF/MEK/ERK) involved in the development of HCC.[2]

The RAS/RAF/MEK/ERK pathway plays an important role in the development of liver cancer. Similar to many signaling networks, this pathway is activated in HCC through a variety of mechanisms. RAS activation is initiated by the binding of extracellular signaling compounds such as hormones and growth factors with VEGFR and PDGFR. The bond between signaling compounds will cause a phosphorylation reaction in the RAS and initiate a series of cascades that lead to the activation of ERK for the proliferation and differentiation and angiogenesis of HCC. Certain mutated components or Ras-Raf-MEK-ERK/MAPK overexpression are increasingly being studied in HCC carcinogenesis. The abnormal target protein signaling pathway contributes to cell proliferation, differentiation, survival, and uncontrolled cell apoptosis is a biomarker of carcinogenic processes.[3]

The PI3K pathway is activated in HCC through various mechanisms such as IGF-1 binding to IGFR thus initiating the P13K signaling series and activating Act which directly influences mTOR which will regulate the proliferation and angiogenesis of HCC.[2]

Barriers from HCC signaling compounds such as MAPK1 (ERK) and Act using special interventions can inhibit the proliferation and angiogenesis of HCC and reduce the concentration of these compounds due to excessive expression due to genetic and epigenetic factors.[4] The use of drugs to block the signaling pathway has been carried out and have reached clinical trials such as Salisarib, Sorafenib, and Selumetinib, but the use of these drugs in the long term will cause side effects such as decreased peripheral blood supply, heart attacks and acute heart failure[3] so that alternative herbal medicine therapy is needed that can inhibit the HCC signaling.

Capsicum annuum L. has natural compounds that can provide enormous benefits for humans and animals. One of the most important compounds in *Capsicum annuum* L. is capsaicin. Capsaicin (8-methyl-N-vanillyl-6-none amid) is an active component of *Capsicum annuum* L. which is irritant to mammals including humans and causes burning and heat in any tissue that is touched. In the pharmaceutical field in addition to relieving pain or pain, capsaicin is also

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known to have anticancer activity. High potential in the pharmaceutical field as anticancer, anti-arthritis and analgesia in addition to having a commercial value in the food industry.[5,6]

Chili (*Capsicum annuum* L.) has a spicy taste and has a very sharp aroma, a spicy flavoring *Capsicum annuum* L. is a capsaicinoid compound. Capsaicinoids include nordihydrocapsaicin, capsaicin, dihydrocapsaicin, nor capsaicin, homodihydrocapsaicin, homocapsaicin, nonivamide.[7] One of the most important compounds in *Capsicum annuum* L. is capsaicin, capsaicin is a secondary metabolite of the *Capsicum annuum* L. In the pharmaceutical field in addition to relieving pain or pain, capsaicin is also known to have anticancer activity because it inhibits certain oncogenic proteins[8]. Further screening of the capsaicin compound against the oncogenic protein produced in the HCC pathogenesis signaling is needed.

Screening components in *Capsicum annuum* L. against MAPK1 and AKT1 target proteins is the initial stage of drug discovery. Screening usually uses computational methods or commonly known as *in silico*. *In silico* consists of various methods including molecular dynamic stimulation, BLAST, and most often used for drug discovery and prediction of drug effects is Molecular Docking.[9] From this background, this study aims to screen in silico the *Capsicum annuum* L. bioactive component of the target protein MAPK1 and AKT1 using the Molecular Docking method to determine the binding energy between the receptor-ligand complex formed and used as a reference for *in vivo* and *in vitro* tests.

MATERIAL AND METHODS

Methods

This study uses a computer-based experimental research design (*in silico*) with the Molecular Docking method.

Sample preparation

3D samples of the protein MAPK1 and AKT1 were obtained through the Protein Data Bank website (www.rscb.org) with GDP ID 3071 for MAPK1 and GDP ID 4EKL for AKT1. For test ligands namely capsaicin obtained through the Pubchem website (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Then both the protein and ligand tests were prepared using the Discovery Studio Visualizer (DSV) application to eliminate native ligands for protein and the conversion of SDF to PDB to continue the docking test. The preparation then continues by removing water molecules and detergents that have the potential to interfere with the course of the reaction so that it affects the value of free energy directly.

Virtual screening and visualization

To find out bond energy and interactions between amino acid residues of oncogenic proteins and capsaicin test ligands, the Autodock 4.0 application was used to determine the bond energy expressed in Gibbs free energy. To find out the interaction of oncogenic protein residues to ligands using PyMOL application for 3D visualization of bonds and Ligplot + for visualization of 2D residues.[9] Validation of the effects of capsaicin was done using the online Pass Web server to determine the value of the antineoplastic activity of the test ligand. If the test has an activity score, then the ligand test is predicted to have a similar effect on the in vitro test and the *in vivo* test.[10]

RESULTS AND DISCUSSION

Molecular docking is research with computational modeling that aims to detect the interaction of a ligand with a receptor. The results of this molecular tethering are in the form of Gibbs free energy and the results of virtual 3D visualization. Gibbs free energy which is considered good when it is negative because it describes the compounds tested by tethering the molecule will attach very well to the receptor and will take place spontaneously. MAPK1 and AKT1 protein bundles and Capsaicin Ligand bundles that were prepared previously in Autodock 4.0 were molecular dockings (Molecular Docking). Vina is an application for docking with output in the form of bond energy from ligand-receptors. Then the ligand file that has very high potential will be visualized using PyMOL, Ligplot + to find out the bond between the ligand and amino acid residues at the binding site and the most potential ligand will be validated using Pass online webserver[10] to find out the potential of the compound.

Bonding energy is influenced by Gibbs free energy (ΔG), the reaction that takes place spontaneously will have free Gibbs energy which is negative at constant temperature and temperature. Bond energy is influenced by several components expressed by the following equation: Δ ΔG⟧ _Hatanic = ⟦ΔG⟧ _Gauss + ⟦ΔG⟧ _Repulsion + ⟦ΔG⟧ _HBond + ⟦ΔG⟧ _Hydrophobic + ⟦ΔG⟧ _Torsion More and more energy components contribute then the value of "G" will be smaller (become negative), the impact of the bond will be stronger and lead to high affinity.[9]

After molecular docking, it was found that Capsaicin binds to MAPK1/ERK with the free energy of Gibbs of -5.5 Kcal/mol and AKT1 of -6.7 Kcal/mol. The free energy of gibbs is so negative that it is ensured that the reaction will take place spontaneously and lead to high affinity. To validate these results an analysis of the binding position of ligands on the amino acid binding site residues using a combination of PyMOL and Ligplot + software and prediction of biological activity using the Pass online Web server to test the potential of compounds as antineoplastic effects will be stated in the Prediction Activity Score.

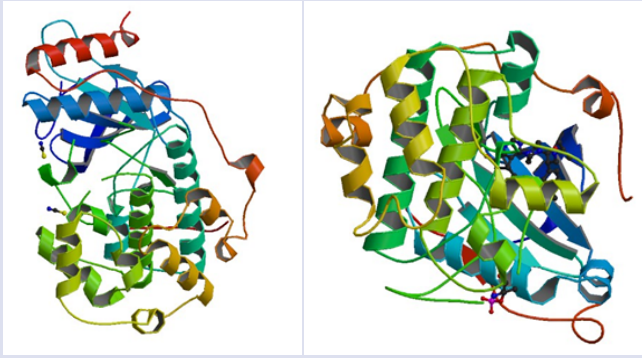

Figure 1: Structure of MAPK1 Oncogenic Proteins (Left) and AKT1 (Right) [https://www.rcsb.org/].

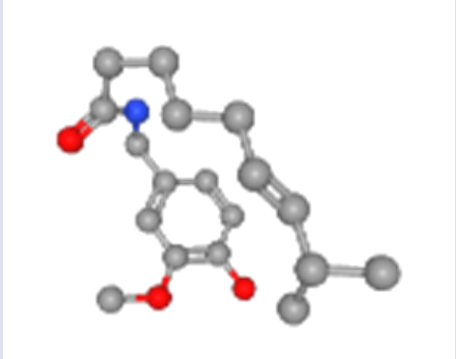

Figure 2: Test Ligand Structure (Capsaicin) [https://pubchem.ncbi.nlm.nih.gov/].

Capsaicin ERK

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-5.5	0.000	0.000
2	-5.4	2.288	3.419
3	-4.9	3.712	4.999
4	-4.9	4.498	6.516
5	-4.7	4.269	6.417
6	-4.7	3.068	4.429
7	-4.5	4.427	6.890
8	-4.5	26.730	29.550
9	-4.3	3.042	4.998

Figure 3: Results of Capsaicin tethering with MAPK1.

Capsaicin Akt1

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.7	0.000	0.000
2	-6.5	1.749	2.443
3	-6.4	4.073	6.505
4	-6.2	3.256	7.886
5	-6.1	3.792	7.502
6	-5.9	3.293	8.261
7	-5.7	3.121	8.405
8	-5.6	5.227	9.232
9	-5.5	29.498	31.669

Figure 4: Results of Capsaicin tethering with AKT1.

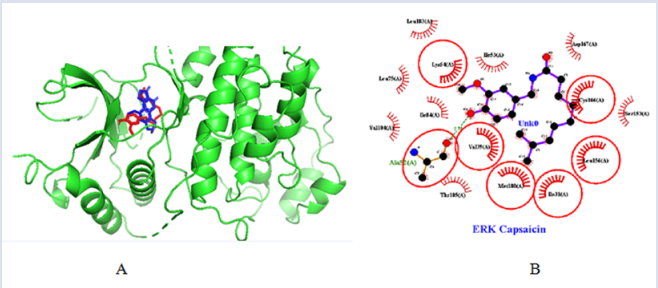

Figure 5: Interaction of ligands and target proteins: A. MAPK1 interactions with CAP (red), B. Amino acid residues and MAPK1 and CAP bonds.

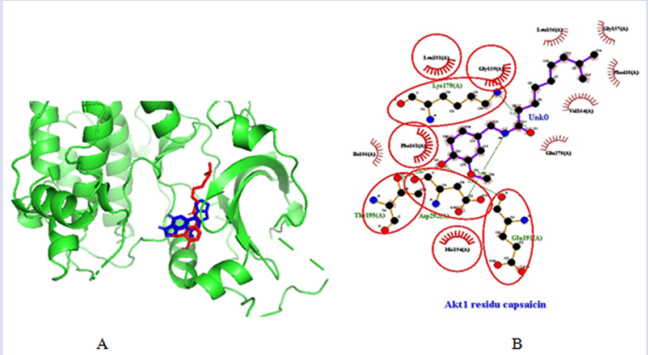

Figure 6: Interaction of ligands and target proteins: A. AKT1 interactions with CAP (red), B. amino acid residues and bonding AKT 1 and CAP bonds.

In the analysis of amino acid residues, Capsaicin to MAPK1 and Capsaicin to AKT1 have relatively similar residues so that it can be concluded that the Binding site of the two compounds approaches the similarity and affects the receptor at a relatively similar place, namely the oncogenic receptor/protein. So that further analysis is needed to determine the effect of Capsaicin on MAPK1 and AKT1. Pass online web server needs to be used to analyze the potential of Capsicum sp. based on their chemical structure.

After testing the activity using the Pass online Webserver with a Potential Activity Score (Pa) score indicator, the capsaicin compound has a Pa 0.690 for preneoplastic treatment, 0.590 for apoptosis agonists and 0.366 antineoplastic activity. The more the Pa value approaches one, the better the potential for activity. Through these tests, capsaicin has good activity value and has the potential as an anti-cancer drug because it inhibits MAPK1/ERK and AKT1 signaling and inhibits the proliferation of cancer cells because it has apoptotic effects of agonists and antineoplastic agents.

CONCLUSION

Based on hs study, capsaicin from *Capsicum annuum* L. has a high affinity for MAPK1 and AKT1 receptor/protein targets with the binding energy of -5.5 Kcal/mol and -6.7 Kcal/mol and Potential Activity Score equal to 0.690 for preneoplastic treatment, 0.590 for apoptosis agonist, and 0.366 for antineoplastic activity and accordingly become candidates for anticancer drugs. Further testing is done through in vitro and in vivo methods as well as standardizing the formulation of capsaicin as an anticancer drug.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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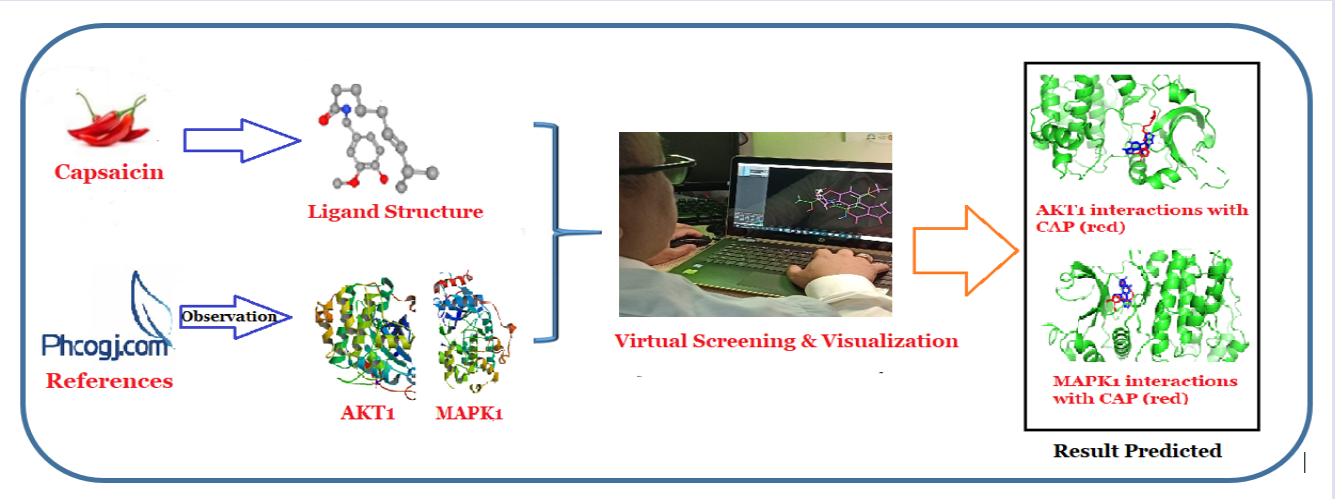
GRAPHICAL ABSTRACT



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Prof. Sri Agus Sudjarwo, Ph.D., DVM., one expert in the field of pharmacology from Universitas Airlangga. He had dreamed of becoming an expert in pharmacology since his childhood because of the many research fields that this science could investigate.

Dr. Mustofa Helmi Effendi, DVM., DTAPH is Associate Professor in Veterinary Epidemiology Head of Department of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University; Head of Halal Research Center, Airlangga University.

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