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In-Silico Studies of Antitumor Activity From Red Ginger (*Alpinia purpurata* Vieill) Bioactive Compounds

Santika Lusia Utami[1,*] Yogi Adhi Nugroho[1] Luthfiana Hardianingtyas[2]
Fatiha Kamilah[2]

[1]*Dept. of Biology, Faculty of Mathematics and Science (FMIPA), Universitas Brawijaya, Malang, Indonesia*
[2]*Dept. of Medicine, Faculty of Medicine. Universitas Indonesia, Jakarta, Indonesia*
[*]*Corresponding author. Email: santikalusia@gmail.com*

ABSTRACT
Many plants from the genus Alpinia are known to have several bioactive compounds that can act as an antitumor in cancer treatments. However, the potency of the bioactive compounds from red ginger as an antitumor agent has not been reported before. This research aims to study the inhibitory activity of several red ginger bioactive compounds against antitumor AKT1 protein through in-silico analysis. In this study, four identified bioactive compounds namely rutin, sitosteryl 3-O-6-palmytoil-β-d-glucoside, kumatakenin, kaempferol-3-o-glucuronide were used as a ligand for molecular interactions. The 3D structure of targetted protein AKT1 (PDB ID: 3O96) was obtained from the PDB database. [6]-shogaol, an antiproliferative and AKT1 inhibitor, was used as a control. In-silico docking analysis is performed in PyRx with the AutoDock Vina program. All bioactive compounds used in this experiment demonstrated good antitumor activity by binding to the inhibitor site of AKT1 protein. Rutin displayed the best potency as an inhibitor to AKT1 with the optimum binding energy of -11,3 kcal/mol as compared to control with -7,4 kcal/mol. These results suggest that bioactive compounds from red ginger may have the potency as an antitumor and can be developed to treat cancer.

Keywords: *AKT1, antitumor, docking, red ginger*

1. INTRODUCTION

Cancer has been a constant battle globally with more than 15 million cases and 9 million deaths in 2018, thus considered as a major cause of mortality worldwide [1]. Cancer is characterized by the continuous multiply of cells in parts of the human body and unable to be controlled or stopped. Tumors are formed as a consequence of the uncontrolled multiplying of cells and have the potential to be metastatic [2]. In cancer, certain intracellular signaling pathways comprising of various kinases and transcription factors are amplificated. This occurance has an implication in the promotion and progression of cancer [3]. Targeting one or more components of an oncogenic signaling cascade is considered as an alternative strategy to prevent the progression of cancer growth.

Several signaling pathways regulate the cellular functions, including P13/AKT signaling pathways comprises of AKT serine/threonine kinase, also known as protein kinase B. AKT is an oncogenic protein that has crucial role in growth, proliferation, survival/apoptosis, transcription, protein synthesis and glycogen metabolism of a cell. The activation of AKT requires the contribution of P13K or phosphoinositide dependent kinases (PDK) as well as growth factors, inflammation and DNA damage. Downstream effectors that involved in this signaling pathway such as mTOR, glycogen synthase kinase 3 beta (GSK3β), or forkhead box protein 01 (FOXO1).

The increasing of cancer cell proliferation and survival in many cancers, including in ovarian, lung, and pancreatic cancers caused by overactivation of AKT [4]. Increased AKT1 activity has been observed in approximately 30-50% of breast, ovarian and

prostate cancers. About 30-40% over activation of AKT2 occured in ovarian and pancreatic cancers [5]. Therefore, targeting AKT could provide a promising strategy for inhibiting cancer growth and therapy.

Many phytochemicals have been reported to inhibit abnormal activated signal transduction pathways, thereby preventing cancer [6]. Red Ginger (Alpinia purpurata Vieill), with its phytochemical compounds such as flavonoid, rutin, kaemprefol-3-rutinoside, kaempferol-3—oliucronide, sitosteryl 3-O-6-palmytoil-β-d-glucoside, kumatakenin, and kaempferol-3-o-glucuronide [7], has long been used as an oriental medicine. There are several researches have been conducted to investigate the anti-inflammatory and chemopreventive activities of these ginger constituents.

Anticancer activity of red ginger including antioxidant, anti-inflammatory, antiproliferative, antiangiogenic, anti-invasive and antimetastatic [8]. Numerous researches in red ginger as anticancer has been conducted for example, 6-gingerol (6-GIN) inhibited cell cycle, proliferation, induced apoptosis in human colorectal cancer cells [9]; 6-Shogaol (6-SHO) is reported to have significant anticancer activity against prostate cancer cells by inhibiting cell survival and inducing apoptosis through reduction of STAT3 and NF-κB activity [10]. This study aimed to analyze the potency of Red Ginger (Alpinia purpurata Vieill) bioactive compounds as an antiproliferative to inhibit the anti-apoptotic protein AKT1 by in silico approach.

2. MATERIALS AND METHODS

2.1 Data preparation

Bioactive compounds of *Alpinia purpurata* Vieill included in this study were *rutin, sitosteryl 3-O-6-palmytoil-β-d-glucoside, kumatakenin,* and *kaempferol-3-o-glucuronide*. The control ligand used in this study was [6]-shogaol. [6] shogaol is a natural compound that acts as antitumor by binding with an allosteric site of Akt protein. The 3D molecular structure of bioactive compounds or ligands were retrieved from PubChem chemical databases. Preparation of ligand was done by minimizing the energy and converting the retrieved data to .pdb format by using Pyrx software.

The structure of Akt1 protein were retrieved from RCSB Protein Data Bank. The retrieved data of Akt1 protein was prepared by deleting water molecule and ligand that attached to the protein, using Discovery Studio 2020 software and saved as .pdb format for further analysis.

2.2 Docking Procedure

Molecular docking simulation was done by using AutoDock Vina program in PyRx software. The maximum grid box value was set. The binding affinity of the protein and ligand interaction was obtained and each result was saved. The saved results were visualized by using Discovery Studio 2020 software to analyze the amino acid residues binding site for each ligand.

Table 1. Comparison among ligands

Ligands	Binding Energy (kcal/mol)	Interactions		
		Hydrogen	Hydrophobic	Van der Waals
Rutin	-11,3	Glu298, Arg273, Asn53, Asn54, Tyr272	Trp80, Leu264, Gln79, Val270, Ile84	Ser56, Val271, Glu85, Cys296, Val83, Ser205, Leu210, Gly294, Asp274, Tyr18
Kumatakenin	-8,9	Asp292, Thr82, Gln79, Asn54	Trp80, Leu210, Leu264, Val270, Lys268	Gly294, Ile84, Lys179, Tyr272, Thr81, Ile290, Thr211, Ser205, Ala212, Leu213
Sitosteryl 3-O-6-palmytoil-β-d-glucoside	- 7,0	Lys297, Phe293	Phe236, Leu295, Met281, Leu156, Val164	Glu278, Lys158, Glu234. Glu298, Tyr229, Thr291, Gly157, Thr160, Gly159
Kaemp ferol	- 10,2	Tyr272, Val271, Asn54, Thr82, Asp292, Ser205	Val270, Leu264, Gln79, Trp80	Ser56, Asn53, Arg273, Asp274, Gly294, Tyr263, Lys268
Shogaol	- 7,4	Gln79	Tyr272, Leu210, Val370, Leu264, Trp80	Thr211. Ser205, Ile290, Lys268, Asp292, Thr82, Thr81

3. RESULTS AND DISCUSSION

AKT1 (gene at 14q32.33) one of three protein-kinase that regulate cellular process in the cells such as proliferations, metabolism and cell growth and angiogenesis. The regulation of AKT1 is through the phosphorylations of various substrate that response to growth factors [11]. Genetic mutations in AKT1 signalling pathway normally led to the induction's oncogenic activities in human cell, malignant glioma and endometrial cancer in some severe cases, non-small lung cancer, melanoma, Hepatocellular carcinoma and breast cancer [12]. In this research we studied several bioactive compounds from red ginger such as rutin, kumatakenin, sitosteryl 3-O-6-palmytoil-β-d-glucoside and kaempferol to evaluate its antitumor activity as an inhibitor to AKT1 protein in cell proliferations and cancer pathway.

Out of four bioactive compound and control used in this experiment, rutin is showed the strongest bond with target protein of AKT1 based on in-silico bioactivity study. Rutin bound to AKT1 with a relatively low binding energy at -11,3 kcal/mol. This compound bound at several sites in AKT1 via hydrogen, hydrophobic and van der waals bonds (Table 1). Rutin bound through hydrogen bonds at 4 sites namely Glu298, Arg273, Asn53, Asn54, Asp292, and Tyr272. Rutin also bound through hydrophobic bond at Trp80, Leu264, Gln79, Val270, Ile84. Low binding energy interactions between rutin and AKT1 may indicate that this compund can easily bound with AKT1. Moreover, rutin also bounds to AKT1 at its active site. The active sites of AKT1 are Ala230, Glu134, Asp292, and Phe161. These sites known as inhibitor site. These bonds are supported by hydrogens bond within those interactions may also indicates that this interaction is relatively stable and likely to deactivate the activity of AKT1 when presences in the cell.

In general, kaempferol also displayed strong bond with AKT1 protein. However, the bond formed within this interaction was not as strong as in rutin. The interactions between kaempferol-AKT1 had -10,2 kcal/mol binding energy. Kaempferol bounds at several sites in AKT1 via hydrogen, hydrophobic and Van der Waals. Kaempferol bound with AKT1 at 5 sites namely Tyr272, Val271, Asn54, Thr82, Asp292, Ser205. It also bound with AKT1 via hydrophobic bound at 4 sites namely Val270, Leu264, Gln79 and Trp80. However, despite having more hydrogen bond interactions, this interaction requires higher binding energy compared to rutin-AKT1. Binding energy can affect the conformational aspects of ligand and protein, also can affects the motion changes in the complex. The flexibility of protein can determine how stable the protein complex [13]. Binding energy of protein-ligands are calculated using low-energy minima [14]. Moreover, the number of active-site interactions between AKT1 and kaempferol are lower than Rutin-AKT1. Kaempferol-AKT1 only had 1 interaction at active site.

Sitosteryl 3-O-6 palmyltoil β-d-glucoside and kumatakenin had weaker interactions and bound to AKT1 compared to rutin and kaempferol. Both compounds had higher binding energy and less amount of hydrogen bond (Table 1). Therefore, this result to an unstable interaction. According to Silverman and Holladay (2014), lack of hydrogen bonds between ligand and protein may indicates a weaker and unstable interactions. This due to the fact that hydrogen bond is a dipole-dipole interaction that formed from a proton group of X---H and normally unbothered by the presence of water.

4. CONCLUSION

AKT1 have several inhibitor sites that can interact with some ligands. Ligands that interacted with the inhibitor sites may have potency to inhibit AKT1 activity and inactivating the protein. Some of the sites are Ala230, Glu134, Asp292, and Phe161. Interactions of several ligands, kaempferol, rutin, sitosteryl, and kumatakenin, with AKT1 protein showed that Rutin displayed best potency as AKT1 inhibitor. Kaempferol, rutin, and kumatakenin binds in the inhibitor sites. However, rutin has binding energy about -11,3 kcal/mol and binds at the inhibition site amino acid residue, Asp292.

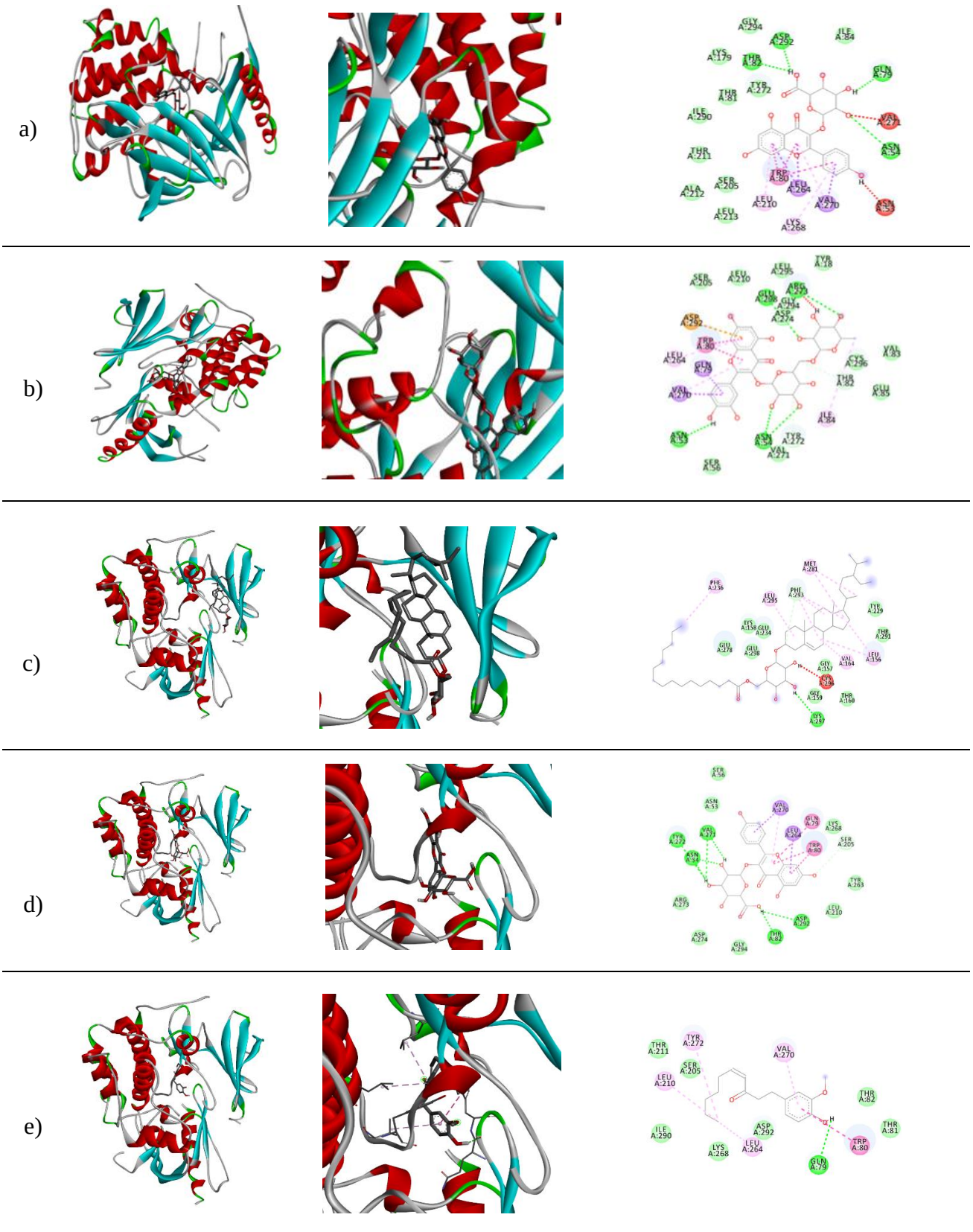

Figure 1. Interactions between ligands; a. Kumatakenin, b. Rutin, c. Sitosteryl 3-O-6-palmytoil-β-d-glucoside, d. Kaempferol, e. Shogaol (Control)

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