Virtual Prediction of Lycopene and Quercetin Effects on Angiogenesis Through VEGFR-2 Pathway

Rizky Senna Samodra^{1*}, Fikriya Novita Sari¹, Setyaki Kevin Pratama¹

¹Department of Biology, Faculty of Mathematics and Natural Science, Brawijaya University, Jalan Veteran, Malang, East Java, Indonesia, 65145

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*Corresponding author: Rizky Senna Samodra; e-mail: risesamodra@gmail.com; tel.: +62-812-6411-8297

ABSTRACT. Angiogenesis is a complex process that is required for cancer cells to perform metastasis. The binding of a growth factor such as VEGF to its receptor is a factor to trigger angiogenesis through the VEGFR-2 pathway. This study analyzed the effect of lycopene and quercetin from watermelon (*Citrullus lanatus*) on angiogenesis through VEGFR-2 pathway. The study was carried out in silico. Ligands were obtained from PubChem and prepared using PyRx, while the protein was obtained from PDB and prepared using BIOVIA Discovery Studio 2019. The docking was carried out by using HEX 8.0.0, and the results were visualized using BIOVIA Discovery Studio 2019. Lycopene and quercetin were able to bind with VEGFR-2 to interrupt the binding of VEGFA. The presence of lycopene and quercetin also lowers the binding strength of VEGFA with VEGFR-2 as they can affect interactions between VEGFA and VEGFR-2 at 4 and 5 amino acid residues by changing the type of interactions to make the binding strength weaker. The binding of lycopene and quercetin potentially interrupted the downstream pathway of angiogenesis through the VEGFR-2 pathway.

Keywords: angiogenesis, cancer, lycopene, quercetin, VEGF-receptor2

INTRODUCTION

Cancer is one of the most dangerous chronic diseases in the world. It is a non-transmittable disease that is one of the main death cause in the world. Cancer patients increase 1,4 out of 1000 people in 2013 to 1,79 out of 1000 people in 2018 [1]. Cancer in Indonesia is placed eighth in southeast Asia, and it affects every people in the society. It does not discriminate whether the people suffer because it is old, young, or whether the gender is male or female [2].

Angiogenesis occurs when local endogenous chemical signals coordinate with endothelial cells and smooth muscles to repair damaged blood vessels or to produce new blood vessels. The angiogenesis process promotes cancer proliferation leading to the metastasis process [3]. Vascular endothelial growth factor (VEGF) is one of the growth factors used in angiogenesis. By binding with its receptor (VEGFR-1, VEGFR-2, VEGFR-3), VEGF stimulates the angiogenesis process utilized by cancer cells to spread to other tissues [4]. Suppressed of VEGFR pathway needs caused cancer cells not to spread to other tissues. The effect of angiogenesis from the VEGFR-2 pathway is much more significant than the VEGFR-1 and VEGFR-3 pathway. Therefore, angiogenesis inhibition is a more useful target for downregulating the VEGFR-2 pathway [5].

Lycopene and quercetin are found in watermelon (*Citrullus lanatus*) with the concentration are 144.27 mg/kg [6] and 4.69-

171.27 µg/g, respectively [7]. Both lycopene and quercetin have anti-inflammatory and antioxidant properties and can suppress VEGFR-2 protein expression [8]. These roles are essential to suppress cancer cell progression from preneoplastic to neoplastic and suppress cancer cell migration [9]. This research is conducted to analyze the effect of lycopene and quercetin on angiogenesis through the VEGFR-2 pathway.

METHODS

Ligand Preparation

Lycopene (CID: 446925) and quercetin (CID: 5280343) were obtained from PubChem database in SDF format. The energy of ligands was minimized and the file format was converted from SDF to PDB format by PyRx software [10].

Protein Preparation

The protein structure was obtained from Protein Data Bank as a VEGFR-2/VEGF-A complex (PDB ID: 3V2A). The protein was then prepared using BIOVIA Discovery Studio 2019 [11] to remove the ligands and water molecules.

Docking and Visualization

Docking was conducted by HEX 8.0.0 software [12] to predict the binding energy and possible ligand interactions and its receptor. Docking results were then visualized using BIOVIA Discovery Studio ver.19 [11].

RESULTS AND DISCUSSIONS

The docking results showed that lycopene has lower binding energy than quercetin with VEGFR-2. The data indicated that lycopene has a higher potential VEGFR-2 inhibitor than quercetin and a stronger binding affinity with VEGFR-2 (Table-1). Unfortunately, when VEGF-A was docked with VEGFR-2-lycopene complex, the binding energy decreased. Lycopene might increase the binding affinity between VEGFR-2 and VEGF-A. Unlike lycopene, quercetin increases binding energy when VEGF-A was docked with VEGFR-2, and this showed that quercetin might decrease the binding affinity between VEGFR-2 and VEGF-A. The lower binding energy indicates that the molecule is more stable than the molecule with higher binding energy [13].

Docking between VEGFR-2 and VEGFA as natural ligands showed 18 amino acid residues consisting of eight hydrogen bonds, three hydrophobic bonds, three electrostatic bonds, and four unfavorable bumps. Hydrogen bonds are a type of strong bond and stronger than hydrophobic and electrostatic bonds. Hydrogen bonds have a high affinity with electrons so that two atoms or different molecules can bind with each other [15]. However, the stability of VEGFR-2 with VEGFA might be affected by 4 unfavorable bumps. Interactions between VEGFR-2 and VEGFA are defined as VEGFR-2 active site.

Lycopene and quercetin that interacted with VEGFA proved a change of interactions between VEGFR-2 and VEGFA. The docking results of VEGFR-2 with lycopene and VEGFA showed 17 amino acid residues interactions that consist of 7 hydrogen bonds, four hydrophobic bonds, an electrostatic bond, and five unfavorable bumps. The presence of lycopene showed a change of binding site between VEGFA and VEGFR-2 in 13 out of 17 amino acid residues, and only four amino acid residues from VEGFR-2 are still binding with VEGFA, which is Tyr137, Lys286, Val219, and Asp257. Furthermore, that complex showed unfavorable bumps with Val219 and Asp257, hydrophobic bond with Tyr137, and hydrogen bond with Lys286.

The docking results of VEGFR-2 with quercetin and VEGFA showed 17 amino acid interactions that consist of a hydrogen bond, eight hydrophobic bonds, an electrostatic bond, and seven unfavorable bumps. The presence of unfavorable bumps might disturb the stability of the interactions, even though there is a change of binding location of VEGFA with VEGFR-2 with the presence of quercetin. The presence of quercetin showed a change of VEGFA binding

site with VEGFR-2 in 12 out of 17 amino acid residues. Only five amino acid residues from VEGFR-2 are still binding with VEGFA, Lys286 with electrostatic bond, Tyr194 and Leu252 with hydrophobic bond, and Asn253 and Asp257 with unfavorable bumps. This also showed that the bond's strength gets weaker after quercetin binds to VEGFR-2 and VEGFA is docked afterward. The results of each bond that occurred from VEGFR-2 and ligands interaction with their binding energy is shown in Table 1.

The interaction of VEGFA with VEGFR-2 is shown in Figure 2. VEGFR2 (gray) is shown from two perspectives to view the VEGFA binding site (yellow). The VEGFA (blue) changes its position in binding with VEGFR-2 when an active compound is present in VEGFR-2. Active compounds covered the active binding site of VEGFR-2 that should be the place that VEGFA. Those data suggested that lycopene and quercetin were possibly interrupted the binding of VEGFA with VEGFR-2.

The VEGFR2-lycopene-VEGFA complex showed that the binding position of VEGFA has a different position than before due to the presence of lycopene (red). Then there is the active site of VEGFR2, which is not covered by VEGFA and changes in VEGFA interaction with VEGFR-2 amino acid (Table 1). Quercetin also changes the binding site of VEGFR2-VEGFA when quercetin interacted with VEGFR2 (Table 1). Both lycopene and quercetin interrupted Val 218 and Val 219 of VEGFR-2 protein and might inhibit VEGFA-VEGFR-2.

Lycopene and quercetin effects on angiogenesis and predicted as a non-competitive inhibitory mechanism. Those compounds bound to non-active sites of VEGFR-2 protein (Table 1). The Val 217, Val 218, and Val 219 residues are active sites of VEGFA-VEGFR-2. after docking with VEGFA. When that amino acid binds with VEGFA, the binding affinity is increased and VEGFR-2 activated. However, interrupted amino acid decreased the binding affinity and disturbed VEGFR-2 [16]. Lycopene bound to the active site of VEGFR-2, while not for quercetin (Table 1). Hence, this phenomenon showed that lycopene and quercetin are potentially inhibited transduction signals for the angiogenesis mechanism through blocking VEGFA-VEGFR2.

The VEGFR-2 structure consists of seven domains, including extracellular regions composed of immunoglobulins (Ig)-like domain. The intracellular region of VEGFR-2 is a tyrosine kinase domain. VEGF-A binds to the second and third extracellular Ig-like domains of VEGFR-2. Ligand binding induces dimerization of the receptor and autophosphorylation. The VEGF ligand binding to domains 2 and 3 of a monomer

receptor increases the probability that the second receptor monomer binds the already bound ligand. Once two receptors are cross-linked with each other, by simultaneous interaction with the ligand,

the domain 7s of Ig-like domain are held close so low-affinity homotypic interactions between domains can stabilize the receptor dimers [17].

Table 1. Types of bonds occurred between ligands and VEGFR-2 and binding energy.

Interaction	Name	Distance (Å)	Category	Type	Energy (kcal/mol)
Lycopene	:LIG1:C - R:PRO166	3.69183	Hydrophobic	Alkyl	-332.00
	:LIG1:C - R:PRO166	5.40581	Hydrophobic	Alkyl	
	:LIG1:C - R:ARG222	3.89126	Hydrophobic	Alkyl	
	:LIG1:C - R:LEU252	3.91698	Hydrophobic	Alkyl	
	:LIG1:C - R:VAL218	3.89939	Hydrophobic	Alkyl	
	R:TYR194 - :LIG1:C	4.93703	Hydrophobic	Pi-Alkyl	
	R:TYR224 - :LIG1:C	4.54714	Hydrophobic	Pi-Alkyl	
Quercetin	:LIG1:H - :LIG1:O	2.21606	Hydrogen bond	Conventional Hydrogen Bond	-169.34
	R:MET213:CE - :LIG1	2.69938	Hydrophobic	Pi-Sigma	
	R:MET213:CE - :LIG1	3.52632	Hydrophobic	Pi-Sigma	
	R:MET213:SD - :LIG1	4.16524	Other	Pi-Sulfur	
	:LIG1 - R:ARG164	4.949	Hydrophobic	Pi-Alkyl	
VEGFR 2-VEGFA	A:TYR39:HH - R:VAL216:O	2.70901	Hydrogen Bond	Conventional Hydrogen Bond	-702.02
	R:VAL218:HN - A:GLU38:O	2.14529	Hydrogen Bond	Conventional Hydrogen Bond	
	A:GLN37:HE22 - R:GLU251:O	2.43555	Hydrogen Bond	Conventional Hydrogen Bond	
	A:PRO40:CD - R:ASN253:OD1	2.97188	Hydrogen Bond	Carbon Hydrogen Bond	
	A:GLY59:HN - R:ASP257:OD1	2.21112	Hydrogen Bond	Conventional Hydrogen Bond	
	R:ASN259:HD22 - A:ARG23:O	2.49472	Hydrogen Bond	Conventional Hydrogen Bond	
	R:SER310:HG - A:CYS68:O	2.60021	Hydrogen Bond	Conventional Hydrogen Bond	
	R:GLY312:HN - A:CYS57:O	2.95978	Hydrogen Bond	Conventional Hydrogen Bond	
	R:VAL219 - A:LEU97	5.28446	Hydrophobic	Alkyl	
	R:ALA308 - A:CYS60	4.1724	Hydrophobic	Alkyl	
	A:VAL69 - R:LEU313	5.25991	Hydrophobic	Alkyl	
	A:GLU73:OE1 - R:TYR137	4.62267	Electrostatic	Pi-Anion	
	R:GLU261:OE2 - A:TYR21	4.89442	Electrostatic	Pi-Anion	
	R:LYS286 - A:ASP34:OD2	4.83518	Electrostatic	Attractive Charge	
	R:SER193:O - A:ASP41:OD2	1.88992	Unfavorable	Unfavorable Bump	
	R:TYR194:CA - A:ASP41:CB	2.067	Unfavorable	Unfavorable Bump	
	R:LEU252:C - A:GLN37:NE2	2.10494	Unfavorable	Unfavorable Bump	
	R:VAL254:N - A:GLN37:NE2	1.49651	Unfavorable	Unfavorable Bump	

Table 1. Continued

Interaction	Name	Distance (Å)	Category	Type	Energy (kcal/mol)
VEGFR2 – Lycopene - VEGFA	R:HIS133:HD1 - A:GLU42:OE2	3.08062	Hydrogen Bond	Conventional Hydrogen Bond	-707.07
	A:SER74:HG - R:VAL254:O	2.63464	Hydrogen Bond	Conventional Hydrogen Bond	
	R:ILE256:HN - A:GLN98:OE1	2.70618	Hydrogen Bond	Conventional Hydrogen Bond	
	R:ASN274:HD22 - A:PRO28:O	1.62579	Hydrogen Bond	Conventional Hydrogen Bond	
	R:LEU277:HN - A:GLU72:OE1	2.28398	Hydrogen Bond	Conventional Hydrogen Bond	
	R:LYS286:HZ1 - A:GLU73:O	2.75082	Hydrogen Bond	Conventional Hydrogen Bond	
	R:THR293:HN - A:GLN22:OE1	2.04705	Hydrogen Bond	Conventional Hydrogen Bond	
	A:TYR39 - R:VAL135	4.62732	Hydrophobic	Pi-Alkyl	
	R:TYR137 - A:MET94	5.37546	Hydrophobic	Pi-Alkyl	
	R:VAL273:CG2 - A:HIS27	3.5305	Hydrophobic	Pi-Sigma	
	R:ARG275 - A:LYS101	3.55749	Hydrophobic	Alkyl	
	R:GLN132:N - A:ASP41:OD1	2.85059	Electrostatic	Attractive Charge	
	R:VAL219:CG2 - A:THR77:OG1	2.17032	Unfavorable	Unfavorable Bump	
	R:ASP257:OD1 - A:GLU30:OE2	5.08976	Unfavorable	Unfavorable Negative-Negative	
	R:LEU272:N - A:GLN22:OE1	1.98483	Unfavorable	Unfavorable Bump;Unfavorable Acceptor-Acceptor	
VEGFR2 – QUERCETIN- VEGFA	R:ASP276:CB - A:GLU72:OE1	1.69256	Unfavorable	Unfavorable Bump	-679.17
	R:PHE288:CE2 - A:ASN100:ND2	2.11485	Unfavorable	Unfavorable Bump	
	R:GLY255:CA - A:GLN37:O	2.4339	Hydrogen Bond	Carbon Hydrogen Bond	
	R:PRO166 - A:ILE29	5.08043	Hydrophobic	Alkyl	
	R:PHE170 - A:VAL20	4.81096	Hydrophobic	Pi-Alkyl	
	R:VAL171 - A:VAL14	5.12477	Hydrophobic	Alkyl	
	A:CYS60 - R:MET191	4.68886	Hydrophobic	Alkyl	
	R:TYR194 - A:ILE29	4.67605	Hydrophobic	Pi-Alkyl	
	A:PRO70 - R:LEU252	5.27598	Hydrophobic	Alkyl	
	A:PRO40 - R:ILE256	4.24375	Hydrophobic	Alkyl	
	R:PHE288 - A:PRO40	5.29418	Hydrophobic	Pi-Alkyl	
	R:LYS286:NZ - A:GLU38:OE2	2.53492	Electrostatic	Attractive Charge	
	R:TYR165:O - A:ILE29:CD1	2.21094	Unfavorable	Unfavorable Bump	

Table 1. Continued

Interaction	Name	Distance (Å)	Category	Type	Energy (kcal/mol)
VEGFR2 – QUERCETIN- VEGFA	R:LYS168:CG - A:ARG23:CB	2.2014	Unfavorable	Unfavorable Bump	-679.17
	R:ARG169:CB - A:VAL15:CG2	2.33676	Unfavorable	Unfavorable Bump	
	R:TYR190:CD1 - A:CYS60:CB	2.18968	Unfavorable	Unfavorable Bump	
	R:ASN253:O - A:GLN37:NE2	2.05063	Unfavorable	Unfavorable Bump	
	R:ASP257:OD2 - A:ASP41:OD1	4.87371	Unfavorable	Unfavorable Negative-Negative	
	R:PHE258:CB - A:ASP41:OD1	1.78168	Unfavorable	Unfavorable Bump	

Figure 1. VEGFR-2 binding with lycopene and quercetin: (A) binding location and ligand interaction; (B) 2D diagram ligand interaction.

VEGFR-2 mediates several physiological and pathological effects of VEGF-A on endothelial cells, such as proliferation, migration, survival, and permeability [17]. The binding of VEGF-A to VEGFR-2 induces proliferation by activation of the extracellular signal-regulated kinases (Erk) pathway. VEGFR-2 stimulates Erk phosphorylation and proliferation by a PKC-dependent pathway that involves the activation of PLC- γ . Meanwhile, on the migration pathway, the binding of VEGF-A with VEGFR-2 mediates cytoskeletal reorganization, migration, and activation of phosphoinositide 3-kinase (PI3K). The activation of PI3K regulates cellular migration by several different growth factors. In the cell survival point of view, activation of PI3K with the generation of membrane-bound PIP₃ results in the membrane targeting and

phosphorylation of protein kinase B (PKB/Akt) and phosphoinositide-dependent kinases 1 and 2 (PDK1 and PDK2). VEGF-A also induces the expression of anti-apoptotic proteins Bcl-2 and A1. Moreover, VEGF-A inhibited apoptosis family members XIAP and survivin that inhibit terminal effector caspases 3 and 7 [17].

Lycopene and quercetin bind to the domain 2 and 3 of the Ig-like extracellular domains of VEGFR-2, suggesting lycopene and quercetin might inhibit receptor dimerization caused by the binding of VEGF-A to domains 2 and 3 of the Ig-like extracellular domains of VEGFR-2. The inhibition of the dimerization process might inhibit the autophosphorylation mechanism and switch off any downstream pathway.

Figure 2. Binding location and interaction VEGFR2 with VEGFA

CONCLUSION

Lycopene and quercetin have the potential to inhibit angiogenesis through the VEGFR-2 pathway. Lycopene and quercetin inhibit VEGFA-VEGFR-2 interaction by blocking VEGFR2. The effect of lycopene and quercetin on VEGFR-2 was the interruption of the active binding site of VEGFA that possibly inhibit any downstream pathway and protein activations required for the angiogenesis process. Further studies are required to understand lycopene and quercetin effect on angiogenesis through the VEGFR-2 pathway by combining with other compounds to formulate an anti-angiogenic drug.

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