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

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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 were 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 VEGF-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 VEGF-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 VEGFR-2–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 |                  |
|             | R:MET213:CE - :LIG1   | 2.69938      | Hydrophobic | Pi-Sigma      | -169.34          |
|             | R:MET213:CE - :LIG1   | 3.52632      | Hydrophobic | Pi-Sigma      |                  |
|             | R:MET213:SD - :LIG1   | 4.16524      | Other      | Pi-Sulfur     |                  |
| VEGFR 2-VEGFA | :LIG1:C - R:ARG164  | 4.949        | Hydrophobic | Pi-Alkyl      |                  |
|             | A:TYR39:HH - R:VAL216:O | 2.70901    | Hydrogen Bond | Conventional Hydrogen Bond |                  |
|             | 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 | -702.02          |
|             | 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 |                  |
| Interaction | Name | Distance (Å) | Category | Type | Energy (kcal/mol) |
|-------------|------|--------------|----------|------|------------------|
| R:His133:HD1 - A:GLU42:OE2 | 3.08062 | Hydrogen Bond | Conventional Hydrogen Bond |
| 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 |
| R:ASP276:CB - A:GLU72:OE1 | 1.69256 | Unfavorable | Unfavorable Bump |
| 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 |
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### Table 1. Continued

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

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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 Bel-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.

| Interaction          | Binding location          |
|----------------------|---------------------------|
| VEGFR2-VEGFA         | ![Binding location](image1) |
| Binding Site of VEGFR2 | ![Binding location](image2) |
| VEGFR2-Lycopene-VEGFA | ![Binding location](image3) |
| VEGFR2-Quercetin-VEGFA | ![Binding location](image4) |

**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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