Potency of active compounds extract of soursop leaves (Annona muricata) as a candidate for cervical cancer drug in Silico

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Abstract. Cervical cancer is a cancer caused by an infection derived from the Human Papillomavirus (HPV) that proliferates in the cervical mucosa. Cervical cancer ranks among the two highest incidences experienced by women, with 23.4% and an average death rate of 13.9%. Vascular endothelial growth factor (VEGF) is an important protein that plays a role in the pathology of angiogenesis in tumor growth and metastasis. Various efforts have been made to minimize mortality from cervical cancer, but they have caused side effects. Indonesia is rich in a diversity of flora that has the potential to be used as a natural medicine, for example, soursop leaves (Annona muricata). The study aimed to predict the compound extract of soursop leaves as a candidate drug for cervical cancer based on a silico approach. The prediction of phytochemical properties is made based on the five laws of Lipinski as seen from the values of absorption, distribution, and metabolism with Swiss ADME. The in-silico method was conducted with the assistance of computer devices, databases, and software like Autodock, PyRx, PyMol, and Discovery Studio. The target proteins used are VEGFR-1 (PDB: 3HNG) and VEGFR-2 (PDB: 3VHE). The results showed that kaempferol, sativene, and thiazole-2,4(3H,5H)-dione, 5-benzylideno-3-[(ethylphenylamino)methyl] meet the five laws of Lipinski. The compound kaempferol has the lowest binding affinity, with a value of -8.5 kcal/mol on VEGFR-1 and -10.2 kcal/mol on VEGFR-2. On the other side, sunitib interactions with VEGFR-1 were -10.0 kcal/mol and -9.4 kcal/mol, respectively. Based on the results, can be concluded that kaempferol has a high potential to be a drug candidate for cervical cancer.

1 Introduction

Cancer is a condition where cells in the body grow uncontrollably, indefinitely, and abnormally [1]. Cancer occurs when normal cells experience very rapid growth, which causes them to be uncontrollable by the body and lose their normal shape [2]. Cancer can appear in various parts of the body, one of which is cervical cancer. According to the Global Burden of Cancer Study (Globucan) data released by the World Health Organization (WHO), the
total cases of cervical cancer in the world in 2020 reached 604,127 cases with a total mortality of 341,831 cases [3]. The incidence of cervical cancer in the world is around 13.1 per 100,000 women [4].

Cervical cancer is cancer that occurs in the female reproductive organs, more precisely the cervix, which is the entrance to the uterus, located between the uterus and vagina [5]. Cervical cancer is the only cancer caused by the Human Papilloma Virus (HPV) [6]. Transmission of the virus can occur through sexual intercourse, especially with frequent changes of partners. Cervical cancer is one of the leading causes of death in women in western, eastern, central, and South African countries.

Various methods have been made to cure cancer such as radiotherapy, surgery, and chemotherapy [7]. However, chemotherapy has a variety of side effects that are bad for the physical and quality of life of cancer patients such as nausea, vomiting, diarrhea, unstable emotions, and damage to the nervous system [8]. Another study [9] reported a protein that is predicted to have the potential to become a candidate protein target for drug attachment and can interfere with mechanisms in cervical cancer, is the Vascular Endothelial Growth Factor (VEGFR) protein. [9] The main function of VEGFR-1 in angiogenesis is important in various pathological conditions, including tumors and vascular diseases such as diabetic retinopathy. Inhibition or modulation of VEGFR1 has become a potential target in the development of therapies aimed at regulating blood vessel growth in various medical contexts [10]. While the main function of VEGFR 2 is as angiogenesis, is to trigger the growth of new blood vessels (angiogenesis) by stimulating the proliferation and movement of endothelial cells to form new blood vessels [11].

Sunitinib is an example of an angiogenesis inhibitor that has been tested to inhibit blood vessel growth in tumors [11]. The drug’s mechanism of action is to inhibit vascular receptor tyrosine kinases and growth factors, which are involved in the process of new blood vessel formation. Of course, the effectiveness and mechanism of action of such compounds in inhibiting angiogenesis in cervical cancer may depend on various factors.

Soursop (Annona muricata L.) is a plant of the dicotyledonae class, annonaceae family, and Annona genus. Soursop is widely found in the Caribbean, Central America, South America, and Asia, especially Indonesia. Soursop leaf extract has shown various anticancer effects such as cytotoxicity, apoptosis induction, necrosis, and proliferation inhibition because it has the main components of annonaceous acetogenin (AGE) and flavonoids. A study conducted [13] through Gas Chromatogram-Mass Spectrometer (GC-MS) testing and analysis of soursop leaf extract, as well as research conducted [14] using a multistage maceration method with a variety of solvents n-hexane (non-polar), ethyl acetate (semi-polar), and methanol (polar) obtained the results of active compounds that were successfully identified and known for their pharmacological performance.

In silico tests are one of the studies that can predict the activity of a compound that has potential as a drug and eliminate other compounds that have low activity [15]. In silico tests have the advantage of saving energy, time, and money [16]. The compounds used must meet the Drug-likeness criteria based on Lipinski's Rule of Five (RO5). Based on this, it is important to innovate natural treatments for patients with cervical cancer that are carried out in silico tests by analyzing potential target proteins and active plant compounds that are considered to have potential as anti-cancer. This study is useful to describe the interaction between active compounds of soursop leaves (Annona muricata L.) against target proteins that play a role in the mechanism of cervical cancer in silico so that it is expected to be used as a reference for information and recommendations in drug discovery exploration efforts made from natural active compounds from plants to treat cervical cancer cases, thus can be a candidate for cervical cancer drug compounds.
2 Method

This research is descriptive research using a computational approach. Data collection techniques using online web databases and literature studies supporting data on interactions between active compounds from soursop leaves (Annona muricata) and target proteins from national and international journals. Data analysis techniques were carried out using the PubChem web database (https://pubchem.ncbi.nlm.nih.gov/). The active compound content of soursop leaves (Annona muricata) was obtained through literature studies based on the results of Gas Chromatography-Mass Spectroscopy (GC-MS) tests conducted [13] and [14]. Then, an analysis of the drug-likeness characteristics of compounds that meet the properties of drug similarity based on Lipinski's rule is carried out. Lipinski's rules include having a molecular weight of 150-500 g/mol, the number of H-bond acceptors < 10, the number of H-bond donors < 5, and the value of the logP partition coefficient < 5. The use of a comparison compound is also needed as a comparison of binding formed from active compounds owned by soursop leaves. The comparison compound used is Sunitinib which is known to have a role as an inhibitor in anticancer mechanisms. Collection of active compound structures and pharmacological analysis through the PubChem web database (https://pubchem.ncbi.nlm.nih.gov/). The compound minimization stage uses PyRx software. Vascular Endothelial Growth Factor Receptor 1 (PDB code: 3HNG) and Vascular Endothelial Growth Factor Receptor 2 (PDB code: 3VHE) target proteins were obtained from lam (https://rcsb.org).

Fig 1. 3D structures of the target proteins used (a) Vascular Endothelial Growth Factor-1 (PDB ID:3HNG), (b) Vascular Endothelial Growth Factor-2 (PDB ID:3VHE)

Molecular docking is a computational procedure that can be used to predict the chemical bond of a macromolecule (receptor) with a small molecule (ligand) efficiently using its structure through molecular docking simulation. The result of docking is the binding affinity value. The more negative the binding affinity value, the stronger the bond and vice versa [17]. Visualization is done using PyMOL and Discovery Studio 2021 software to see the docking results to be representative. Based on the visualization results, it is known the type of bond and the amount of similarity of amino acid residue attachment formed between the active compound of water hyacinth leaves and the comparison compound against certain cervical cancer target proteins.

3 Results and Discussion

In pharmaceutical science, not all compounds can be used as medicines. It is necessary to predict the physiochemical properties of medicines (drug-likeness) according to Lipinski's
Rule of Five (RO5). The Lipinski requirement consists of a molecular weight not greater than 500 Da, does not have a partition coefficient value (logP) more than 5, the number of hydrogen bond donors less than 5, and hydrogen bond acceptors less than 10 [18].

The Lipinsky rule can analyze the physicochemical properties of a ligand to determine the hydrophobic or hydrophilic properties of a compound to pass through the cell membrane by passive diffusion. The log P value indicates a solubility coefficient in fat or water with a range of -0.4 to 5, because molecule weights greater than 500 Da cannot diffuse through cell membranes. The greater the Log P value, it means the molecule is more hydrophobic. Molecules with high hydrophobic properties tend to have higher levels of toxicity because they will last longer on bilayer lipids and will be distributed more widely within the body so that the selectivity of binding to the target enzyme becomes reduced [19]. On the other side, negative log P values are also not good because the molecule cannot pass through the bilayer lipid membrane. The number of hydrogen binding donors and receptors describes the higher the hydrogen bonding capacity, the greater the energy required for the absorption process to take place. In general, the Lipinski rule describes the solubility of certain compounds to penetrate cell membranes by passive diffusion [20].

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW (150-500) g/mol</th>
<th>HBA (&lt;10)</th>
<th>HBD (&lt;5)</th>
<th>MLogP (&lt;5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caryophyllene</td>
<td>204</td>
<td>0</td>
<td>0</td>
<td>4.73</td>
</tr>
<tr>
<td>Cyclohexane-1,2-</td>
<td>172</td>
<td>4</td>
<td>2</td>
<td>0.96</td>
</tr>
<tr>
<td>Dicarboxylic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiazole-2,4(3H,5H)dione, 5-</td>
<td>338</td>
<td>2</td>
<td>0</td>
<td>2.07</td>
</tr>
<tr>
<td>benzylideno3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sativene</td>
<td>204</td>
<td>0</td>
<td>0</td>
<td>4.27</td>
</tr>
<tr>
<td>Methyl 9oxononanoate</td>
<td>186</td>
<td>3</td>
<td>0</td>
<td>2.08</td>
</tr>
<tr>
<td>Isopulegol</td>
<td>154</td>
<td>1</td>
<td>0</td>
<td>2.74</td>
</tr>
<tr>
<td>2-(Dimethyl.lambda.(4)-</td>
<td>192</td>
<td>4</td>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>sulfanylidene)malonic acid, dimethyl ester</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>286</td>
<td>6</td>
<td>4</td>
<td>2.28</td>
</tr>
</tbody>
</table>

Table 1. Drug-likeness properties based on the rule of five Lipinski of active compound extract of Annona muricata [13] and [14].

Based on twenty-two compounds extract of soursop (Annona muricata) leaves that have been obtained through literature studies from the research [13] and [14], computational chemical studies have been carried out to find out the characteristics of drug-likeness of compounds that have potentially as a drug for cervical cancer. According to Table 1, there are 8 compounds obtained that has meet drug-likeness properties according to Rule of Five Lipinski: Caryophyllene, Cyclohexane-1,2-Dicarboxylic Acid, Thiazole-2,4dione, 5-benzylideno-3-, Sativene, Methyl 9-oxononanoate, Isopulegol, 2-(Dimethyl-lambda. (4) sulfanylidene) malonic acid, dimethyl ester, and kaempferol. These compounds can penetrate the cellular lipid membranes by passive diffusion and do not require much energy at the time of absorption [21].
Table 2. Result of molecular docking between selected active compound extract of *Annona muricata* and the target protein

<table>
<thead>
<tr>
<th>Target Proteins</th>
<th>Ligands</th>
<th>Binding Affinity (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vascular Endothelial Growth Factor Receptor 1</em> (VEGFR-1)</td>
<td>Sutinib (Control)</td>
<td>-10.0</td>
</tr>
<tr>
<td></td>
<td>Kaempferol</td>
<td>-8.5</td>
</tr>
<tr>
<td></td>
<td>Sativene</td>
<td>-7.6</td>
</tr>
<tr>
<td></td>
<td>Thiazole-2,4(3H,5H)dione, 5-benzylideno-3</td>
<td>-7.6</td>
</tr>
<tr>
<td><em>Vascular Endothelial Growth Factor Receptor 2</em> (VEGFR-2)</td>
<td>Sutinib (Control)</td>
<td>-9.4</td>
</tr>
<tr>
<td></td>
<td>Kaempferol</td>
<td>-10.2</td>
</tr>
<tr>
<td></td>
<td>Sativene</td>
<td>-7.7</td>
</tr>
<tr>
<td></td>
<td>Thiazole-2,4(3H,5H)dione, 5-benzylideno-3</td>
<td>-7.3</td>
</tr>
</tbody>
</table>

The molecular docking process produces a binding affinity value between the test compound and the target protein used. By comparing the value binding affinity from test results with comparative compounds, the binding energy value is obtained which indicates the amount of energy required by the compound to form a bond between the ligand and the receptor. The more negative of binding affinity value, the stronger the bond is formed [18]. On the other side, if the active compound’s attachment position with the control compounds has great or close similarities, then the composite is assumed to be more stable in interacting and affecting the activity of the target protein [22].

According to the results of molecular docking in Table 2, there are 3 selected compounds that have the highest binding affinity of the other 5 compounds, these compounds are Kaempferol, sativene, and Thiazole-2,4(3H,5H)-dione, 5-benzylideno-3. The control compound used as a comparison is Sunitinib. Sunitinib is a drug used in cancer therapy and includes the class of tyrosine kinase inhibitors. This drug works by inhibiting a variety of tyrosine kinase enzymes, including Vascular Endothelial Growth Factor Receptor (VEGFR) and Platelet-Derived Growth Factor Receptor (PDGFR). Through this mechanism, sunitinib helps inhibit the growth of new blood vessels and slows down tumor growth in certain types of cancer.

Vascular endothelial growth factor (VEGF) is an important protein that plays a role in angiogenesis pathology in tumor growth and metastasis, macular degeneration, diabetic renoprotective, and inflammatory processes. VEGFR1 and VEG FR-2 interact with each other for a signal transduction process, which begins with the activation of biochemical pathways in the endothelial cells that trigger the proliferation, migration, and differentiation of the endothelial cell, as well as the production of proteolytic enzymes that help in the expansion of blood vessels [23]. VEGFR-1 is a negative regulation of VEGFR-2 when it binds to VEGF-A. VEGFR-2 raises VEGFR-1 suppression of endothelial cell proliferation [24]. VEGFR-1 regulates angiogenesis by mechanisms that involve ligand-trapping, receptor homo- and heterodimerization. VEGFR-2 is the primary VEGF receptor and plays a role in stimulating the processes of angiogenesis, vasculogenesis and affecting vascular permeability and endothelial cell proliferation, which is important for normal blood vessel growth [25]. The mechanisms that govern VEGFR-2 activation, its ability to recruit signaling proteins and to undergo downregulation are highly regulated by its carboxyl terminus [26].
Fig 2. Compound bond visualization 2,4(3H,5H)-dione, 5-between (a) Sunitinib (b) Kaempferol, (c) Sativene, and (d) Thiazole benzylideno-3 with target protein VEGFR-1

Fig 3. Compound bond visualization between (a) Sunitinib (b) Kaempferol, (c) Sativene, and (d) Thiazole-2,4(3H,5H)-dione, 5- benzylideno-3 with target protein VEGFR-2
On the target protein of VEGFR-1, kaempferol had the most negative binding affinity value than the other compounds, with a bonding affinity value is -8.5, followed by sativene of -7.6 and Thiazole-2,4(3H,5H)-dione, 5-benzylideno-3 of -7.6. The three compounds that have been mentioned have a binding affinity value that is not much different from the drug compound sutinib -10.0. It can be said that the three of these composites have the potential to bind with VEGFR-1 so that there is an interaction between VEGF-A and also VEGFR-2. On the other side target protein of VEGFR-2.

VEGFR 2 kaempferol has a binding affinity value of -10.2 which is more negative than sutinib of -9.4 as a drug comparison, which means the compound kaempferol can be the best candidate as a drug for cervical cancer. Then other compounds such as satineve with a value of -7.7 and Thiazole-2,4(3H,5H)-dione, 5-benzylideno-3 of -7.3 also has good potential to be used as a cervical cancer drug. According to these binding affinity values, especially kaempferol when strongly bound to VEGFR-2 can inhibit the presence of the bond between VEGFR-2 and VEGF-A so that there is no angiogenesis in tumor growth and metastasis of cancer cells.

The Kaempferol location bond with VEGFR-1 protein has similar controls of, as many as, 9 amino acid residues and 3 similar types of binding. The first type of bond is the hydrophobic bond that has the same amino acid residues with controls including VAL 841, ALA 859, VAL 909, VAL 892, LEU 882, and CYS 1039. Hydrophobic bonds play an important role in keeping the structure of proteins stable. Although hydrophobic bonds tend to be weaker than covalent or ionic binding, but they remain important because they contribute to the stability of protein structures and the formation of other biological structures [27]. The second type of binding, hydrogen, has the same amino acid residues as GLU 910 and ASP 1040. Hydrogen bonds are a type of intermolecular interaction that is stronger than hydrophobic bonds [28]. Hydrogen bonds also play a very important role in the structure and stability of proteins. The third is the electrostatic, hydrophobic bond with the same amino acid residues with control LYS 861. It ends with the halogen, hydrophobic bond with the amino acid residue LEU 833. Halogen bonds also include weak bonds. The implantation of Kaempferol with VEGFR 1 protein tends to be weak but many have hydrophobic and hydrogen bonds both contributing to the stability and shape of the overall three-dimensional protein. Hydrogen bonds form secondary structures such as the alpha helix and the beta-sheet, while the hydrophobic bond forms the hydrophobic nucleus and helps maintain the larger protein structure [29]. Both interact with each other and contribute to the stability and overall function of proteins.

On the other side, the sit bond of the sativene compound with the VEGFR-1 protein has similarities with the control of as many as 7 amino acid residues and 2 similar types of binding. The first type of bond is that hydrophobic bonds have the same amino acid residues with controls including VAL 909, VAL 892, ALA 859, LEU 1029, VAL 841, CYS 1039, and electrostatic, hydrophobic links with LYS 861. Sativene has many hydrophobic bonds so it can form and maintain the structure and function of proteins. Thiazole-2,4 (3H,5H)-dione and 5-benzylideno-3 compounds with the VEGFR 1 protein have a similar control of as many as 1 amino acid residue and 1 type of bond is the same type of hydrophobic bond, according to the LEU 882 amino acid residue.

Kaempferol with VEGFR-2 protein has similar controls of as many as 6 amino acid residues and 2 similar types of binding. The first type of bond is a hydrophobic bond that has the same amino acid residues with controls including LYS868, PHE 1047, VAL 848, LEU 1035, and ALA 866. This hydrophobic bond occurs away from the liquid environment and tends to group within the globular structure of the protein [30]. In addition, there are also hydrogen bonds that have one residual of the same amino acid with the control covering ASP 1046. The hydrogen bond between kaempferol with the same amino acid residues on sutinib.
indicates the same type of interaction, which in this case describes the similarity of activity. Hydrogen bonds play an important role in the interaction between proteins and compounds. The accumulation of kaempferol with the VEGFR-2 protein tends to be weak but there are many hydrophobic bonds. Hydrophobic bonds help maintain the stability of the three-dimensional structure of proteins. The hydrophobic part of the protein interacts with the hydrophobic portion of the compound, forming a stable hydrophobic bond [31]. Hydrophobic bonds contribute to the formation of stable molecular complexes and affect their nature and function.

The sativene compound has similar controls of as many as 8 amino acid residues and 2 similar types of binding with the target protein VEGFR-2. The first is the hydrophobic bond. The same amino acid residues with controls include VAL 899, LEU 1035, LYS 868, VAL 916, PHE 1047, and VAL 848. And, the second bond is Hydrophobic Other. The same amino acid residue with control is CYS 1045. These bonds are other intermolecular interactions such as hydrogen bonds, ionic binding, electrostatic interactions, and Van der Waals styles that involve hydrophobic bonds. It influences the structure and function. In Thiazole-2,4(3H,5H)-dione, 5-benzylideno-3 with VEGFR 2 protein has a similar control of as much as 1 amino acid residue and 1 type of binding is the same type of hydrophobic bond with LEU 889 amino acid residue.

4 Conclusion

In this study, there are three active compounds of soursop leaves that have potential as candidates for cervical cancer drugs: Kaempferol, Sativene and Thiazole-2,4(3H,5H)-dione, 5-benzylideno-3. These three compounds have drug-likeness properties based on Rule of Five of Lipinski. Kaempferol is the most significant and stable compound as an inhibitor for target proteins of VEGFR-1 and VEGFR-2. So, it can be concluded that Kaempferol is the highest potential candidate for cervical cancer drugs. The result of this study needs to be deepened with further research related to bioavailability, feasibility, and toxicity to the human body before being applied as an alternative to cervical cancer drugs.

Acknowledgement

The author would like to thank the friends of the bioinformatics study group (NARAYA), Department of Biology Universitas Negeri Surabaya who always support the researchers. Also, to LPPM Universitas Negeri Surabaya as the funder of this research so that this article can be made. Thanks to DAPT-EQUITY Program, Lembaga Pengelola Dana Pendidikan (LPDP), Ministry of Finance, Indonesia for supporting this publication.

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