Investigation of Moringa Leaf Compounds as Colon Anticancer Agents Using Bioinformatics and Molecular Docking Methods

Dwi Yulia Saputri1, Triana Arum Kusumaningtyas2, Prismo Bagas Setiadi3, Rifki Febriansah4

1,2,3 Pharmacy Study Program, Faculty of Medicine and Health Sciences, University Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183
Email: dwiyulasaputri@gmail.com1; trianaarumk@gmail.com2; prismo431@gmail.com3; rifki.febriansah@umy.ac.id4

ABSTRACT

Introduction - Colon cancer is a disease that has a high mortality rate. Cancer treatments such as chemotherapy can cause severe side effects. So, it is necessary to do research on herbal plants to minimize these side effects. The bioactive compounds in Moringa leaves (Moringa oleifera L.) are known to have antioxidant and anticancer activity, so they have the potential to be used as cancer treatment.

Purpose – . This study aims to determine the potential of Moringa leaves as an anticancer agent in silico.

Methodology/Approach – The in silico test used is STITCH-STRING bioinformatics and molecular docking. STITCH-STRING bioinformatics was used to determine the target protein of the test compound, and molecular docking was used to determine the binding affinity between the test compound and cancer protein.

Findings – In the STITCH-STRING bioinformatics test, the potential targets for kaempferol and quercetin were TP53-ESR1 and STAT3-EGFR, respectively. Test Molecular docking on cancer protein showed that quercetin compound in EGFR protein had the best binding with a docking score of -9.2 kcal/mol. This shows good results because the docking score obtained is lower than the comparison drug (Fluorouracil) which has a docking score of -4.2 kcal/mol. These results indicate that compounds in Moringa leaves (Moringa oleifera L.) have the potential to be used as anticancer agents.

Originality/ Value/ Implication – Moringa leaf research using bioinformatics and molecular docking methods is still rarely done, so this research can be used as further knowledge related to the search for moringa leaf compounds that have the potential for anticancer using bioinformatics and molecular docking methods.

Keywords: anticancer, Moringa leaves, bioinformatics, molecular docking

INTRODUCTION

Cancer is a disease with a prevalence rate of death that is increasing every year. This is caused by uncontrolled cell growth in the body (Afifah, 2018). Colon cancer is a type of cancer that attacks the large intestine, which sufferers often ignore because of its slow development. The cancer treatment business in Indonesia has grown rapidly, such as chemotherapy, surgery and radiotherapy. Chemotherapy is a cancer treatment by killing cancer cells through the administration of special drugs. However, this treatment has several disadvantages, namely severe side effects due to the use of very strong drugs that not only kill cancer cells, but also attack healthy cells. Therefore, it is necessary to carry out new innovations through the utilization of the potential of natural ingredients to minimize side effects.

Indonesia is a country with the second highest level of biodiversity after Brazil, which means that Indonesia's biodiversity is very abundant. Moringa oleifera L. or often known by the public as Moringa is one of the plants that are widely distributed in tropical areas such as Indonesia. According to research, Moringa leaves contain a class of polyphenolic compounds such as flavonoids and phenolic acids. Based on research by Vergara-Jimenez, et al (2017) the main flavonoids in Moringa leaves are quercetin, kaempferol and myrecytin. The content of quercetin compounds in Moringa leaves is a strong antioxidant (Hardiyanti, 2015). The content of kaempferol in Moringa leaves is an antioxidant and anticancer compound that can reduce the risk of cancer.

This paper will discuss the content of kaempferol and quercetin compounds in Moringa leaves which have potential as anticancer based on in silico studies. Moringa leaf research using bioinformatics and molecular docking methods is still rarely done, so this research can be used as further knowledge related to the search for moringa leaf compounds that have the potential for anticancer using bioinformatics and molecular docking methods. Previous studies have discussed the activity of Moringa leaves as an anti-inflammatory agent on 6COX cyclooxygenase-2 protein using the molecular docking method (Purwanto et al, 2021). The purpose of this study was to determine the antioxidant and anticancer activity based on the bioactive compounds found in Moringa leaves. The results of this study are expected to provide scientific evidence regarding the potential of bioactive compounds from Moringa Oleifera L as an anticancer agent of the colon based on bioinformatics and molecular docking methods.
LITERATURE REVIEW
Colon Cancer
Colon cancer is a type of cancer that attacks the large intestine which is often overlooked because of its slow development. In the early stages, patients do not feel serious pain and severe complaints so that when they enter an advanced stage, treatment becomes difficult (Mangan, 2009). According to Wijayakusuma (2010), risk factors for cancer include:
- Having a history of gastrointestinal disorders and being >40 years old
- History of congenital disease
- Ulcerative colitis
- Patients with polyposis or family members suffering from polyposis

Symptoms of colon cancer include: flatulence, pain and tension, abdominal part If palpated there is a bulge, decreased appetite, bleeding from the rectum and blockage and narrowing of the large intestine to the anus which causes difficulty in defecation (Mangan, 2009).

Moringa oleifera L.
In Indonesia, the moringa plant is known by various names. In Sulawesi it is known as wori, kelol, kelor or kero. In Madura it is called maronggih. In Malay and Sundanese it is called moringa. In Aceh it is called Merong. Ternate is called kelol. In Sumbawa it is called kwahna and in Minang it is called munggai (Hardiyanti, 2015). In the systematics (taxonomy) of plants, the moringa plant (Moringa oleifera is classified as follows:
Regnum: Plantae (Plants)
Division: Spermatophyta
Sub division: Angiospermae
Class: Dicotyledoneae
Sub class: Dialeptalae
Order: Rhoeidales (Brassicales)
Family: Moringaceae
Genus: Moringa
Species: Moringa oleifera

(Roloff et al, 2009)

The content in Moringa leaf (Moringa oleifera L) includes vitamin A, vitamin B, vitamin C, potassium, calcium, protein and iron that are beneficial to the body as well as more than 40 antioxidants for traditional medicine (Krisnadi, 2010). According to research, the results of the analysis of ethanol extract in Moringa leaves showed the content of flavonoid compounds, steroids, alkaloids, terpenoids, tannins, carbohydrates, saponins, glycosides and reduced sugars. (Hazani, 2014).

In a previous study conducted by (Laksmsiani et al, 2019) regarding quercetin compounds in breast cancer, it was found that quercetin has an affinity for HER-2 protein with a binding energy of -8.24 kcal/mol. The mechanism of quercetin is the formation of hydrogen bonds in the HER-2 protein which can inhibit HER-2 in breast cancer. Another study conducted by (Putri, PVP et al, 2019) found that the potential as an in silico colorectal anticancer agent was obtained from quercetin compounds with bond energy values of -9.54 kcal/mol (COX-2) and -4.59 kcal/moles (Capase-3).

METHOD
Tools and Materials
Tools
used in this research are several software which includes Discovery Studio Visualizer, Marvin Sketch, Autodock Tools and Autodock Vina.

Materials
The materials used in this study were colon cancer proteins (TP53, ESR1, STAT3, EGFR) and SARS-Cov2 (3CL-Pro and SARS-Cov2 Protease) proteins which can be downloaded at https://www.rcsb.org/, the structure of compounds in Moringa leaves (kaempferol and quercetin) and comparison compounds (5-fluorouracil) which can be downloaded at https://pubchem.ncbi.nlm.nih.gov/.

RESEARCH PROCEDURE
STITCH-STRING Bioinformatics Method
In the process of finding the target protein, it begins by opening database on the http://stitch.embl.de/ page on the internet which is used to search for direct target proteins from the active compounds of Moringa leaves. Enter the name of the test compound in the search menu, after which its interaction with various types of target proteins will be known. After the Direct Target Protein is obtained, the test is continued by opening database on the https://string-db.org/ page on the internet to search for the Indirect Target Protein of the test compound. Then proceed with the search for proteins that can regulate the proliferation process in colon cancer on the NCBI website (https://www.ncbi.nlm.nih.gov). Proteins that regulate the occurrence of the proliferative process in colon cancer are taken from the NCBI web. Then proceed with the determination of proteins that can interact with compounds in Moringa leaves and colon cancer using a Venn Diagram. Venn diagrams between direct protein targets, indirect protein targets, and proteins that regulate proliferation in colon cancer were created using Venny.2.1 (http://bioinfogp.cnb.csic.es/tools/venny/) (Hermawan et al., 2021). The overlapping protein was considered as the target protein of each compound and continued with visualization using Cytoscape and then two target proteins were selected from each compound based on the highest degree score that would be used for molecular docking.

Molecular Docking Method
In this method started by downloading several applications such as Autodock Vina and other supporting applications, namely Discovery Studio Visualizer. Download the target protein target on the Bank's GDP web with PDB format. The
target protein to be tested is a protein associated with proliferation in colon cancer obtained from the STRING bioinformatics method. The ligand native be extracted directly from file target protein. The protein and ligand were converted into file PDBQT Autodock Tools. After getting the file in PDBQT form, it is continued by running it using autodock vina to get the RMSD results. To determine the RMSD value, select the conformation with an RMSD value of less than 2. Visualization of the docking to see the bond between the protein and ligand the test Discovery Studio Visualizer application.

RESULT AND DISCUSSION
Test In Silico Bioinformatics Method with STITCH-STRING
After searching through the NCBI web, 1611 genes were found that can trigger the proliferation process of colon cancer cells. Direct Target Protein (DTP) and Indirect Target Protein (ITP) from flavonoid compounds, namely kaempferol and quercetin contained in Moringa leaves were obtained as much as 38 proteins and 49 proteins. After that, analysis was carried out on the Direct Target Protein and Indirect Target Protein using a Venn diagram with the help of Venny 2.1 on the internet. The slice results from the Venn diagram will be continued in the Cytoscape application for analysis to see the degree score. Then visualize the results with Cytoscape based on degree score that has been obtained. Below is the result of visualizing the top protein target with the Cytoscape.

Table 1. Visualization of Results Based on Degree Score

<table>
<thead>
<tr>
<th>No.</th>
<th>Protein</th>
<th>Degree score</th>
<th>Protein</th>
<th>Degree score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>TP53</td>
<td>25</td>
<td>TP53</td>
<td>32</td>
</tr>
<tr>
<td>2.</td>
<td>ESR1</td>
<td>23</td>
<td>STAT3</td>
<td>32</td>
</tr>
<tr>
<td>3.</td>
<td>HSP90A1</td>
<td>19</td>
<td>EGFR</td>
<td>31</td>
</tr>
<tr>
<td>4.</td>
<td>MAPK3</td>
<td>19</td>
<td>MYC</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>CREB1</td>
<td>18</td>
<td>HRAS</td>
<td>26</td>
</tr>
<tr>
<td>6.</td>
<td>AHR</td>
<td>16</td>
<td>PTGS2</td>
<td>25</td>
</tr>
<tr>
<td>7.</td>
<td>MAPK1</td>
<td>16</td>
<td>KRAS</td>
<td>25</td>
</tr>
<tr>
<td>8.</td>
<td>RXRA</td>
<td>15</td>
<td>MTOR</td>
<td>24</td>
</tr>
<tr>
<td>9.</td>
<td>SRC</td>
<td>15</td>
<td>CASP3</td>
<td>24</td>
</tr>
<tr>
<td>10.</td>
<td>CYP1A1</td>
<td>14</td>
<td>INS</td>
<td>23</td>
</tr>
</tbody>
</table>

After visualization of the results based on the degree score was obtained, the target protein was then selected to be used for binding affinity analysis using the molecular docking. Kaempferol compounds will be linked to TP53 and ESR1, and Quercetin will be linked to STAT3 and EGFR proteins.

Test In Silico Method Molecular Docking
The molecular docking test aims to describe the occurrence of interactions between the ligand molecule and the target protein in a computational way. The test in this study was carried out to link the ligands of the test compound to the target protein of colon cancer. The test results on each compound with its target protein showed an interaction between quercetin and EGFR protein, quercetin with STAT3, kaempferol with TP53, and kaempferol with TP53.

Table 2. results Docking with cancer cell protein

<table>
<thead>
<tr>
<th>Compound Test</th>
<th>Protein Target</th>
<th>RMSD Value</th>
<th>Docking Score</th>
<th>Conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaempferol</td>
<td>TP53</td>
<td>-8.6</td>
<td>1.201</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>ESR1</td>
<td>1.629</td>
<td>Quercetin</td>
<td>2</td>
</tr>
<tr>
<td>0.867</td>
<td>STAT3</td>
<td>-7.3</td>
<td>-9.2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>EGFR</td>
<td>1.852</td>
<td>1.508</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>TP53</td>
<td>1.829</td>
<td>-4.8</td>
<td>2</td>
</tr>
<tr>
<td>-Fu</td>
<td>ESR1</td>
<td>-5.1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>STAT3</td>
<td>1.512</td>
<td>-4.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>EGFR</td>
<td>1.778</td>
<td>-4.2</td>
<td>6</td>
</tr>
</tbody>
</table>

Based on table 2, the quercetin-EGFR bond has the best affinity with a docking of -9.2 kcal/mol. Comparative test was performed with 5-Flourouracil linked to four target proteins and showed docking than kaempferol and quercetin compounds, as shown in table 2. Quercetin compounds have a better binding affinity for target proteins compared to standard chemotherapy drugs. In previous studies it was also mentioned that quercetin compounds also have a good affinity for the 6COX receptor (Purwanto et al, 2021).
CONCLUSION AND RECOMMENDATION

Based on the STITCH-STRING bioinformatics test, potential cancer protein targets with kaempferol compounds are TP53 and ESR1 with degree scores of 25, respectively, and 23. In the case of quercetin, the potential cancer proteins were STAT3 and EGFR with a degree score of 32 and 31. In the molecular docking, the cancer protein EGFR had the best bond with quercetin compounds with a docking of -9.2 kcal/mol. This shows good results because the docking obtained is lower than the comparison drug (Fluorouracil) which has a docking of -4.2 kcal/mol. As for supporting the sustainability of this research, further tests are needed such as in vitro and in vivo order to add further evidence that kaempferol and quercetin compounds in Moringa leaves can be potential anticancer agents.

REFERENCES


