ISSN 2961-7758

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

Dwi Yulia Saputri[1], Triana Arum Kusumaningtyas[2], Prismo Bagas Setiadi[3], Rifki Febriansah[4]
[1,2,3,4] Pharmacy Study Program, Faculty of Medicine and Health Sciences, University Muhammadiyah Yogyakarta, Yogyakarta, Indonesia, 55183
Email : dwiyuliasaputri@gmail.com[1] ; trianaarumk@gmail.com[2] ; prismo431@gmail.com[3] ; rifki.febriansah@umy.ac.id[4]

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 (Afiyah, 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 (Hardiyanthi, 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.

ISSN 2961-7758

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, kelo, keloro 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 kelo. In Sumbawa it is called kawona and in Minang it is called munggai (Hardiyanthi, 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 : Dicotyledone
Sub class : Dialypetalae
Order : Rhoeadales (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 (Laksmiani 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

ISSN 2961-7758

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

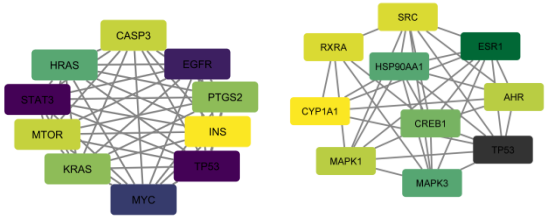
Test *In Silico* Bioinformatics Method with STITCH-STRING

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*

No.	Kaempferol		Quercetin	
	Protein	*Degree score*	Protein	*Degree Score*
1.	TP53	25	TP53	32
2.	ESR1	23	STAT3	32
3.	HSP90AA1	19	EGFR	31
4.	MAPK3	19	MYC	30
5.	CREB1	18	HRAS	26
6.	AHR	16	PTGS2	25
7.	MAPK1	16	KRAS	25
8.	RXRA	15	MTOR	24
9.	SRC	15	CASP3	24
10.	CYP1A1	14	INS	23

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.



(1) (2)

Figure 1. Visualization of bioinformatics test results for quercetin (1) and kaempferol (2)

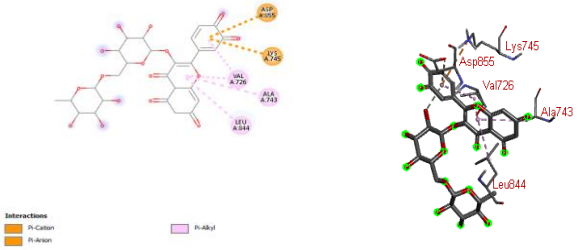
Test *In Silico* Method *Molecular Docking*

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

Compound Test	Protein Target	RMSD Value	Docking Score	Conformation
Kaempferol	TP53	-8.6	1.201 - 6.8	4
	ESR1	1.629	Quercetin	2
0.867	STAT3	-7.3	-9.2	3
	EGFR	1.852	1.508	6
-Fu	TP53	1.829	-4.8	2
	ESR1	-5.1	2	5
	STAT3	1.512	-4.0	4
	EGFR	1.778	-4.2	6

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-Flououracil 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).



(1) (2)

ISSN 2961-7758

Figure 2. 2D (1) and 3D (2) molecular docking of EGFR against quercetin

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

Afiyah, R. K. (2018). Dukungan Keluarga Mempengaruhi Kemampuan Adaptasi (Penerapan Model Adaptasi Roy) Pada Pasien Kanker Di Yayasan Kanker Indonesia Cabang Jawa Timur. *Journal of Health Sciences*, *10*(1), 96–105. https://doi.org/10.33086/jhs.v10i1.15

Hardiyanthi, F.: Pemanfaatan Aktivitas Antioksidan Ekstrak Daun Kelor (*Moringa oleifera*) dalam Sediaan Hand and Body Cream. *Skripsi*, Fakultas Sains dan Teknologi UIN Syarif Hidayatullah, Jakarta, 2015.

Hardiyanthi, F.: Pemanfaatan Aktivitas Antioksidan Ekstrak Daun Kelor (Moringa oleifera) dalam Sediaan Hand and Body Cream. *Skripsi*, Fakultas Sains dan Teknologi UIN Syarif Hidayatullah, Jakarta, 2015

Hazani, K.F. (2014). Pengaruh pemberian ekstrak etanol daun kelor (*Moringa oleifera* L.) terhadap kadar Malondialdehide (MDA) dan kualitas spermatozoa epididimis mencit (*Mus musculus* L) yang dipapar timbal (Pb) asetat.*Undergraduate thesis*, Universitas Islam Negeri Maulana Malik Ibrahim Malang.

Hermawan, A. et al. 2021. 'Bioinformatics and in vitro studies reveal the importance of p53, PPARG and notch signaling pathway in inhibition of breast cancer stem cells by hesperetin', *Advanced Pharmaceutical Bulletin*, 11(2), pp. 351–360. doi: 10.34172/apb.2021.033.

Krisnadi AD. 2010. *Kelor Super Nutrisi*. Blora: Pusat Informasi dan Pengembangan Tanaman Kelor Indonesia

Mangan, Y. (2009). *Solusi sehat mencegah & mengatasi kanker*. Jakarta: Agromedia Pustaka.

Purwanto, D.S., Susanti, H., & Sugihartini, N. (2021). Docking Molekuler Potensi Anti Inflamasi Quersetin Daun Kelor (*Moringa oleifera* L.) dengan Autodock-Vina. *Jurnal Ilmiah Manusia dan Kesehatan*. Vol 4, 309-313

Putri, P. V. P., Susanti, N. M. P., & Laksmiani, N. P. L. (2019). Senyawa Kuersetin sebagai Agen Antikanker Kolorektal secara In Silico. *Jurnal Kimia*, 13(2): 166–171.

Rastini, M.B.O., Giantari, N.K.M., Adnyani, K.D., and Laksmiani, N.P.L. (2019). Molecular docking aktivitas antikanker dari kuersetin terhadap kanker payudara secara *in silico*. *Journal of chemistry.* 13(2): 180-184.

Roloff A., Weisgerber, H., Lang, U. et al. (2009). *Moringa oleifera* Lam, *Enzyklopädie der Holzgewächse*, vol. 40, pp. 1 – 8.

Vergara-Jimenez, M., Almatrafi, M. M., dan Fernandez, M. L. (2017). Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. *Antioxidants*. 6 (91):1