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An In Silico Evaluation of Flavonoids in *Allium* sp. as a Potent Anti-Acute Myeloid Leukemia (AML) Through FLT3 Inhibition

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ABSTRACT. *Acute Myeloid Leukemia (AML) is the most common leukemia caused by genetic abnormalities that affect the hematological proliferation and induce incomplete differentiation of myeloid cells in the peripheral blood and bone marrow. AML involving FLT3 gene mutation results in overexpression of FLT3 receptors. This study intended to predict the interaction between the FLT3 receptor in AML with flavonoids found in Allium sp. by a molecular docking method. Several 3D molecule structures of the highest flavonoids from Allium sp., including quercetin, kaempferol, isorhamnetin, myricetin, fisetin, morin, isoquercetin, quercitrin, spiraeoside, and quercetin-3,4-O-diglucoside were obtained from the PubChem database. Meanwhile, the FLT3 receptor complex with gilteritinib 3D structure was obtained from the RCSB PDB database. All compound properties were assessed using Swiss-ADME. FLT3 receptor prepared using Discovery Studio 2019. Meanwhile, all flavonoids' energy as ligands is minimized using PyRx 0.8 software. The specific docking was performed using Autodock Vina integrated with PyRyx v 0.8 software and visualized using Discovery Studio 2019, and several parameters of the docking data were analyzed. All flavonoids show favorable drug-likeness and pharmacokinetics properties apart from four quercetin derivatives. Meanwhile, the molecular docking result shows that Quercetin, kaempferol, isoquercetin, quercitrin, spiraeoside, and quercetin-3,4-O-diglucoside have inhibitory potential against FLT3 receptors because they share several same binding sites as control. They also have a fairly high binding affinity compared to the other compounds tested. Molecular dynamic simulation result demonstrates a considerably stable complex. It is estimated that quercetin and kaempferol from Allium sp. have the potential to be used as therapeutic drugs against AML.*

Keywords: *allium, AML, flavonoids, FLT3, molecular docking*

INTRODUCTION

Blood cancer or leukemia is one of the diseases with a high mortality rate, whereas Acute Myeloid Leukemia (AML) has a low survival rate, with a value of less than 20-30 percent. Acute Myeloid Leukemia (AML) is the most common type of leukemia, especially in adults, responsible for around 80% of all leukemia cases [1, 2]. This cancer is characterized by genetic abnormalities, chromosomal translocations, or molecular alterations that disrupt hematological proliferation and induce incomplete differentiation of myeloid cells in the peripheral blood and bone marrow. As a result, blood manufacturing will be disrupted [3, 4, 5]. Fms-like Tyrosine Kinase (FLT3) is one of the mutated genes in AML patients. This gene is detected in approximately 30% of AML patients. FLT3, a type III receptor kinase, is abundantly expressed in hematopoietic cells and plays critical functions in cell survival and proliferation. Inhibition of FLT3 signaling is one technique for treating AML in this aspect [6, 7].

The protein FLT3 is encoded on chromosome 13q12. FLT3 is a protein that plays a role in the differentiation and refinement of hematopoietic precursor cells, which will subsequently differentiate into several types of blood cells. As an outcome, uncontrolled hematopoietic precursor cell production might lead to blood cancer. When CD34+ cells possessed substantial levels of the ligands, the activity of these cells reduced FLT3 expression. Furthermore, the presence of dimers in the plasma membrane, which results in autophosphorylation, limits FLT3 expression. Overexpression of the FLT3 receptor occurs when CD34 cells lose their positive and become ligand-independent [8,9].

Allium is part of the Liliaceae family that grows across the world. *A. sativum, A. fistulotum, A. cepa, A. ursinum,* and others are among the over 700 species in this genus. These plants are generally used as food ingredients, household

spices, and even traditional medicine. The main beneficial compound inside these plants is flavonoids. Flavonoids consist of phenolic compounds varied in structures and have anti-allergic, anti-inflammatory, antiviral, antioxidant, anti-cancer, anti-microbial, and antidiarrheal properties. Importantly, flavonoids are proven to be an anti-cancer agent by inhibiting the activated receptor [10, 11, 12]. The most common flavonoids of *Allium* sp. are quercetin, kaempferol, isorhamnetin, myricetin, fisetin, morin and their derivatives. The most common derivatives are Quercetin-3,4-*O*-diglucoside, and Quercetin-4'-*O*-glucoside (spiraeoside) account for more than 85% flavonols in onion [13]. Latest report on isoquarcetin and quercitrin identified them as active flavonols that plays an important role as anti-cancer, and cardioprotectant via oxidative stress modulation [12].

Based on the information presented and the probability of flavonoid compounds as an anti-cancer agent, an in-silico investigation was conducted. This research aims to predict the interaction between the FLT3 receptor in AML with flavonoids found in Allium sp. by a molecular docking method [14]. The expected interaction between the potential compounds and the receptor is an inhibitory interaction.

RESEARCH METHODS

Sample Retrieval

Flavonoids in *Allium* sp. were chosen as the main compound for this paper. Of all the flavonoids contained in *Allium* sp., quercetin (PubChem CID: 5280343), kaempferol (PubChem CID: 5280863), isorhamnetin (PubChem CID: 5281654), myricetin (PubChem CID: 5281672), fisetin (PubChem CID: 5281614), morin (PubChem CID: 5281670), isoquercetin (PubChem CID: 5280804), quercitrin (PubChem CID: 5280459), spiraeoside (PubChem CID: 5320844), and quercetin-3,4-O-diglucoside (PubChem CID: 5320835) are among those with the highest percentage [12]. The compounds' 3D structures were obtained from the PubChem database, downloaded in SDF format. Gilteritinib was used as a control in this study. Another compound used in this research as control is Gilteritinib. This compound is a known inhibitor for FLT3 and 78 other kinases, thus potentially effective for AML [15]. Furthermore, the FLT3 3D structure (PDB ID: 6JQR) was downloaded from the RCSB PDB database in .pdb format.

Compound Screening

The characteristics assessment of flavonoids was done by Swiss-ADME (Absorption, Distribution, Metabolism, and Excretion) analysis. Swiss-ADME (http://www.swissadme.ch/) is a website that can predict specific compound properties and their pharmacokinetics related to their efficiency and effectiveness as drugs. Results obtained from this analysis are lipophilicity, hydrophilicity, physicochemical properties, drug-likeness, pharmacokinetic, medical chemistry, and the estimation of gastrointestinal or blood-brain barrier permeation of potential compounds [16].

Allium sp. flavonoid compounds bioactivity then predicted using PASS-online web server. PASS- online predict the possible bioactivity by opposing the compound's structure-activity relation to another identical compound with known bioactivity [17]. All listed compounds were analyzed and shown anticancer activity with various Pa value. Pa value indicates the similarity of all tested compounds to the known drugs, higher Pa value tends to show higher resemblance to the known drugs, while lower Pa value known to be synonymous to lower bioactivity.

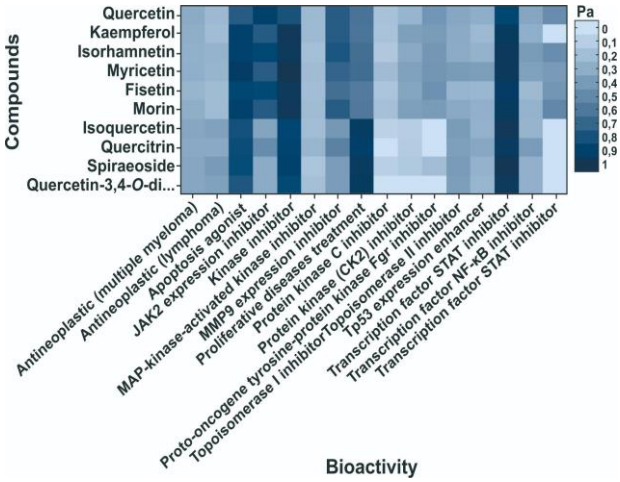


Figure 1. Probable Bioactivities of *Allium sp.* Bioactive Compound.

Sample Preparation

The FLT3 structure was downloaded from the RCSB PDB database (www.rcsb.org) and was prepared by Discovery Studio 2019. Protein preparation was carried out to remove water molecules and native Ligand. Protein preparation is to ensure sample purity and avoid other compound that could potentially interfere with the docking process [18]. Furthermore, the structure of the 3D ligands ware downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format, then minimized its energy and converted to PDBQT format with Open Babel integrated with PyRx 0.8 software [19].

Molecular Docking and Dynamic

The protein and ligands docking were performed using Autodock Vina integrated with PyRyx v 0.8 software in a specific docking area

(Dimension X: 13,6821; Y: 21,9159; Z: 13,)5448 and Center X-28,804; Y: 1,2803 Z: -14,4508). The ligand molecules were positioned within the target protein's binding region. The docking result was taken from the model 0 of all the 9 available model. A scoring formula was utilized to rate the various postures and ligands based on the docking pose findings [20]. Discovery Studio 2019 was used to visualize the docking results in better representation. Several parameter values of the docking data, including binding affinity values, binding locations, and interaction amino acid residues on the ligands, were also examined [21].

Molecular dynamic simulations were performed using CABS-Flex 2.0 web server [22]. The simulation parameters were set following the default settings. The fluctuation plot during 50 cycles simulation then downloaded in .csv format and visualized using Microsoft Excel in the form of line graph. The molecular docking simulation were meant to predict the stability of protein-ligand complex after docking. The stability of the complex indicated by the RMSF value in the Angstrom (Å) units. The value between 1-3 Å represent a stable complex during simulation period [23].

RESULTS AND DISCUSSIONS

Drug-Likeness

A screening test for ten potential compounds, quercetin, kaempferol, isorhamnetin, myricetin, fisetin, morin, isoquercetin, quercitrin, spiraeoside, and quercetin-3,4-O-diglucoside was done by Swiss-ADME analysis. Based on the database result, all compounds passed the first parameter of Lipinski's rule of five, that is, having a molecular weight of less than 500 Dalton. Another parameter is the m Log P value less than 5, which is covered by all parameters. According to literature [24], a potential compound for drugs must have hydrogen acceptor and donor bonds equal to or less than 10 and 5, respectively. Five of ten compounds have hydrogen donor bonds of more than five and causes a violation of Lipinski's rule of five [25, 26]. These compounds are myricetin, isoquercetin, quercitrin, spiraeoside, quercetin-3,4-O- diglucoside. These compounds have a low GI absorption value. Meanwhile, the GI absorption value of other compounds was high. The value of GI absorption is influenced by the hydrophilicity and solubility of a compound, which can be predicted using Lipinski's five rule assessment [27]. Most of the compounds have a good bioavailability of 0.55. However, there are four compounds that have a low bioavailability of 0.17. Good bioavailability scores can be obtained based on flexibility and polarity. Meanwhile, compounds that are too flexible and polar will have low bioavailability scores [28,29].

Based on the results (Table 1.), it is known that most compounds pass Lipinski's rule of five criteria. Lipinski's rule of five is composed of parameters needed to assess the drug-likeness of specific compounds. Meanwhile, bioavailability score and GI absorption are parameters of the compound's pharmacokinetics [28]. However, despite the one violation and low GI absorption of myricetin, this compound is still used in the following analysis. The reason is that this compound still more likely has the potential to become a promising drug [30].

Molecular Docking

Gilteritinib is Food and Drug Administration (FDA) approved drug that has the ability to inhibit multi receptor tyrosine kinase, one of them is FLT3 that overexpressed during AML progression. Gilteritinib reduces the phosphorylation of STAT5 to 0%, AKT to 9%, and ERK to 1% based on the control in human AML cells. Moreover, gilteritinib inhibited cell growth in cells expressing FLT3 mutants exogenously [31]. These factors support the use of gilteritinib as control in this study. Gilteritinib has a binding affinity of -8,8 kcal/mol with 7 hydrogen bonds and 4 hydrophobic interactions (Table 2).

The docking results show that quercetin is predicted to have the ability to inhibit FLT3 receptor activity. The binding affinity of quercetin is -8,7 kcal/mol with five hydrogen bonds and eight hydrophobic interactions (Table 2). Quercetin binds to several same residues as gilteritinib, such as CYS 694, TYR 693, ALA 642, dan LEU 818 in hydrophobic interaction, and ASP 829 in a hydrogen bond. Quercetin and gilteritinib have same binding cavity in FLT3 receptor as seen in docking visualization result as both of them superimpose (Fig. 1B). Quercetin is one of the unique compounds with potential in cancer treatment. In favorable concentrations, quercetin can suppress the tumorization process in several cancer pathways. Some of them are leukemia, melanoma, breast, head, neck, lung and ovarium cancer [32].

Isoquarcetin, quercitrin, quercetin-3,4-*O*-diglucoside, and spiraeoside are quercetin derivatives that are also predicted to have potent inhibitory activity toward FLT3 receptor. The docking result of these flavonols indicates positive result. They have considerably high binding affinity (Table 2) and same binding pocket as gilteritinib (Fig. 2), gilteritinib as control depicted in yellow. Isoquarcetin has four hydrogen bonds which of them are CYS 694, LEU 616, GLU 692,

GLY 617 and three hydrophobic interactions that are LEU 818, VAL 624, ALA 642. Some of those residues superimpose with gilteritinib residue at CYS 694, LEU 818, and ALA 642. Study shows that Isoquercetin plays an essential role in the Wnt signaling pathway which are correlated with several types of cancer including colorectal and breast cancer, leukemia, and some other cancer [33]. As for quercitrin, there are four hydrogen bonds and six hydrophobic interactions formed. TYR 693 and ALA 642 are residues that exhibit the same interaction as gilteritinib. The most dominant derivatives, Spiraeoside and Quercetin-3,4-*O*-diglucoside, also signify promising results. Spiraeoside serves promising results as it is bound to the same six residues as gilteritinib which are CYS 694, TYR 842, PHE 830, LEU 818, and ALA 642. Quercetin-3,4-*O*-diglucoside is as good. In addition to its high binding affinity (-8,4 kcal/mol), it is also bound to several same residues as Gilteritinib which are CYS 694, ALA 642, and LEU 818. For comprehensive docking results, check Table 2.

Another compound that has the potential to be FLT3 inhibitor is kaempferol. The binding affinity of kaempferol is -8,7 kcal/mol. The docking result shows that kaempferol has the same binding cavity as gilteritinib (Fig. 2B). Kaempferol and FLT3 interaction formed one hydrogen bond and seven hydrophobic interactions, those are CYS 694 and LEU 616, ALA 642, VAL 624, LEU 818, CYS 828, VAL 675, LYS 644, respectively. Some residues with the same interaction as gilteritinib are CYS 694, ALA 642 and LEU 818. The similarity between kaempferol-FLT3 and gilteritinib-FLT3 can be seen in the visualization result (Fig 1C). According to literature [34], kaempferol is a typical cytotoxic chemotherapeutic agent for AML treatment. This compound can damage the DNA of abnormal cells.

Flavonoids such as isorhamnetin, myricetin, fisetin, and morin are commonly found in the onion family of plants. These four flavonoids were docked, and their inhibition of FLT3 receptors does not have the same binding site as gilteritinib-FLT3. Overall, the flavonoids isorhamnetin [35], myricetin [36], fisetin [37], and morin [38] may inhibit numerous receptors in cancer, which may contribute to their anti-proliferative, pro-apoptotic, and anti-metastatic activities. However, because it did not bind at the same place as gilteritinib as a control, the docking analysis between myricetin, fisetin, and morin with FLT3 protein revealed no inhibitory action.

Table 1. Drug-likeness and pharmacokinetics screening results of potential compounds based on Lipinski's rule of five by Swiss-ADME analysis

Flavonoid	PubChem Id	Formula	Parameter Lipinski				Violation	Bioavailability	GI Absorption
			MW (g/mol)	mLogP	nON	nOHNH			
Quercetin [12]	5280343	C15H10O7	302,24	-0,56	7	5	0	0,55	High
Kaempferol [12]	5280863	C15H10O6	286,24	-0,03	6	4	0	0,55	High
Isorhamnetin [12]	5281654	C16H12O7	316,26	-0,31	7	4	0	0,55	High
Myricetin [12]	5281672	C15H10O8	318,24	-1,08	8	6	1	0,55	Low
Fisetin [12]	5281614	C15H10O6	286,24	-0,03	6	4	0	0,55	High
Morin [12]	5281670	C15H10O7	302,23	-0,58	7	5	0	0,55	High
Isoquercetin [13]	5280804	C21H20O12	464,38	-2,59	12	8	2	0,17	Low
Quercitrin [13]	5280459	C21H20O11	448,38	-1,84	11	7	2	0,17	Low
Spiraeoside [13]	5320844	C21H20O12	464,38	-2,59	12	8	2	0,17	Low
Quercetin-3,4-O-diglucoside [13]	5320835	C27H30O17	626,52	-4.62	17	11	3	0,17	Low

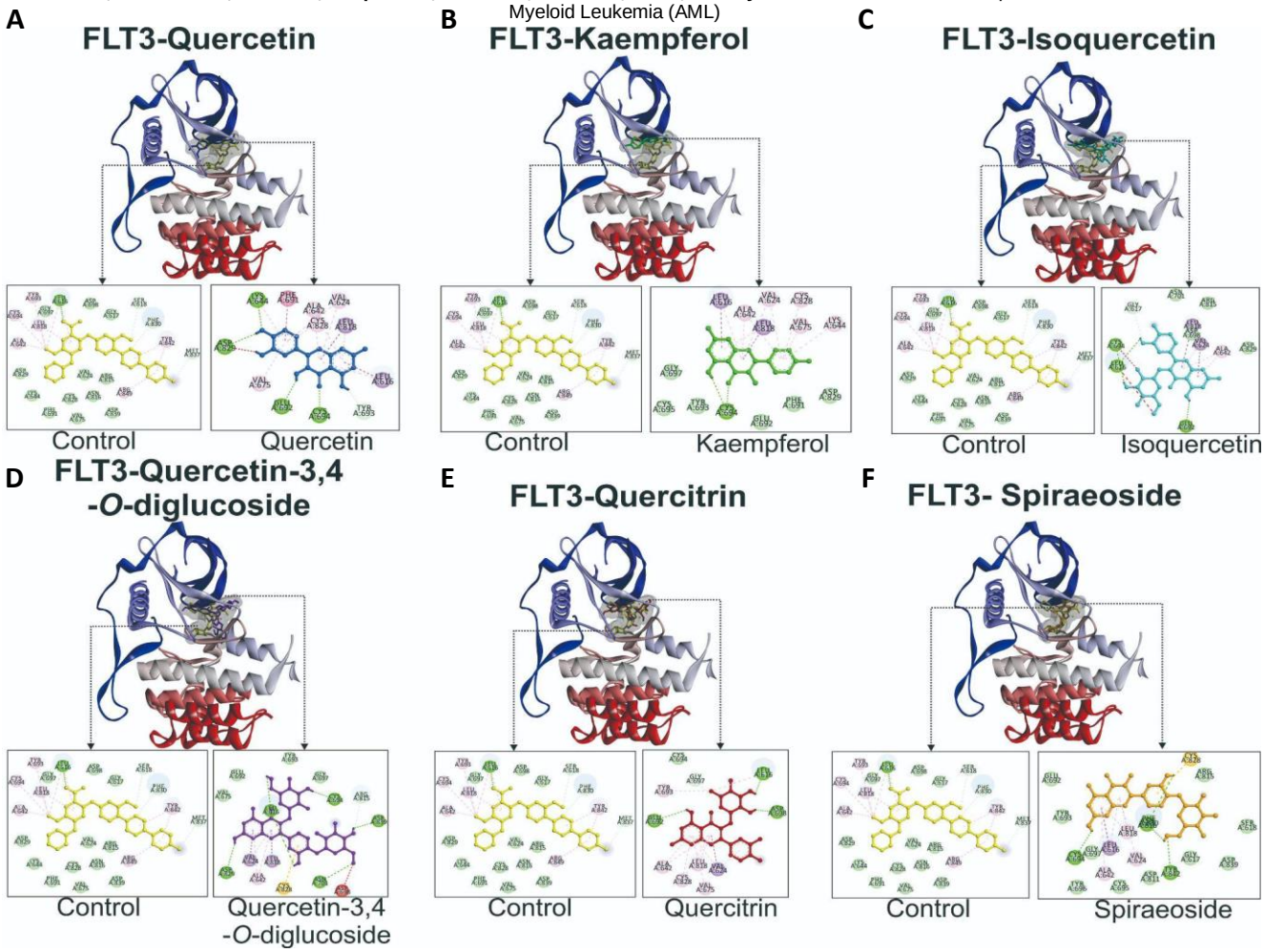


Figure 2. Docking result visualization between protein (FLT3) and ligand, A) Quercetin; B) Kaempferol; C) Isoquercetin; D) Quercetin-3,4-O-diglucoside; E) Quercitrin; F) Spiraeoside

Table 2. The docking results in detail

Ligand	Binding affinity (kcal/mol)	Chemical interaction	
		Hydrogen Bond	Hydrophobic interaction
Inhibitor (Gilteritinib)	-8,8	LEU 693, PHE 830, MET 837, SER 618	LEU 818, TYR 693, CYS 694, ALA 642, TYR 842, ARG 849
Quercetin	-8,7	GLU 692, CYS 694, ASP 829, LYS 644, TYR 693	PHE 691, ALA 642, VAL 624, CYS 828, VAL 675, LEU 818, LEU 616, LYS 644
Kaempferol	-8,7	CYS 694	LEU 616, ALA 642, VAL 624, LEU 818, CYS 828, VAL 675, LYS 644
Isoquercetin	-7,5	CYS 694, LEU 616, GLU 692, GLY 617	LEU 818, VAL 624, ALA 642
Quercitrin	-8,4	GLU 692, LEU 616, ASP 698, GLY 697	TYR 693, ALA 642, CYS 828, LEU 818, VAL 675, VAL 624
Quercetin-3,4-*O*-diglucoside	-8,4	CYS 694, LEU 616, ASP 839, ASN 701, ASP 829, ARG 815	VAL 624, ALA 642, LEU 818, CYS 828
Quercetin-4'-*O*-glucoside (Spiraeoside)	-8,3	CYS 694, TYR 842, PHE 830, ASN 816	LEU 616, LEU 818, VAL 624, ALA 642, CYS 828
Isoharmnetin	-6,5	GLU 858, SER 930, SER 585, ARG 931	GLU 862
Myricetin	-6,6	SER 930, PHE 928, GLU 858, GLU 862, SER 585, ARG 931	PHE 928, ARG 933
Fisetin	-6,6	SER 585, PHE 928, ASP 586	ASP 586, ARG 933
Morin	-6,5	SER 930	ASP 586, GLU 862, PHE 928

Molecular Dynamic

The molecular dynamic simulation results exhibit slight conformational stability between receptor-control and receptor- flavonoid complex. [23] stated that RMSF value between 1-3 Å indicate complex stability during simulation period. Hence, most amino acid in each complex were relatively stable, some of the highest fluctuation pointed and labelled (Fig. 3), fluctuation lower than control not labelled, even if the RMSF value considerably high. FLT3-Quercetin complex shown in Fig. 3A possess two unstable amino acid namely LYS 709 and ASN 781 with fluctuation of 4,284Å and 5,827Å respectively. The conformation of the FLT3-Kaempferol have three unstable amino acid namely SER 633, ASN 781 and MET 837 with RMSF value 3,597Å; 5,079Å; 3,723Å respectively. FLT3- Isoquercetin complex have one significant fluctuation at MET 837 with RMSF value 5,393Å. FLT3- Quercetin-3,4-*O*-diglucoside complex are actually quite stable with considerably low fluctuation, most unstable amino acid was ASN 781 and MET 837 with fluctuation of 3,502Å and 3,182Å. The conformation of FLT3 and Quercitrin have three unstable amino acid and the fluctuation peaked at the end of the cycle. SER 585 have RMSF value of 4,165Å; TYR 889 have RMSF value of 3,698Å; and the highest was GLU 951 with 5,705 Å. FLT3- Spiraeoside complex showed quite stable RMSF value with the highest fluctuation being 3,752Å of SER 762 residue. The only unstable residue that may affect the stability of the protein was MET 837 found in kaempferol, quercetin-3,4-*O*-diglucoside and isoquercetin since the residue was in the active site of the protein, regardless of that, the RMSF value always below 4 Å.

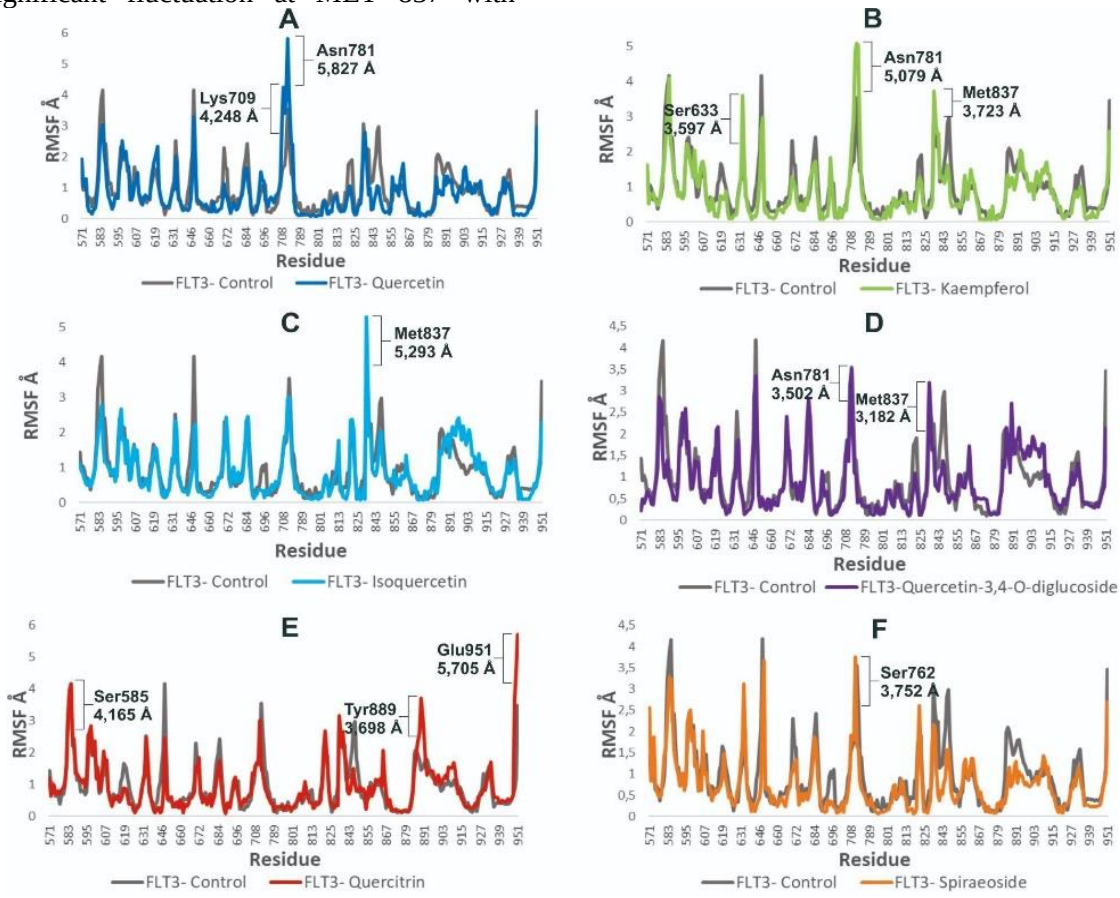


Figure 3. Molecular dynamic simulation result analyzed by CABS-Flex web server; A) Quercetin; B) Kaempferol; C) Isoquercetin; D) Quercetin-3,4-O-diglucoside; E) Quercitrin; F) Spiraeoside

Discussion

Allium sp. is the most common herb in Indonesian households and is used in Indonesian food because of its strong smell and taste. Those characteristics are suitable with the Indonesian cuisine that has strong taste because of various herbs and spices [39]. The abundance usage of *Allium sp.* in Indonesian country specially for daily ingredients of cooking also supported by the fact Indonesia has a mass number of garlic and shallot production with affordable price by local people. Based from Indonesian Statistic Centre's website in 2020 Indonesian farmers produce approximately 1.815.445 kg of onions and 81.805 kg. In addition to its abundance, onions and other cultivars from the species of *Allium* sp. offers high economic value for the society [40,41,42]. However, the climate factor may affect its abundance, still, the amount of yields is more than enough to supply the market [43]. Indonesian

strategic geography supporting optimal growth of every plant and ensuring the optimal and abundance of active compound from the *Allium sp*. [39].

Beside of the usage on Indonesian daily cook, many Indonesian ancestor believe *Allium sp*. has various medicinal benefit based from empirical evidence and sometimes processing it into *Jamu*, an Indonesian traditional herbal medicine [44]. Scientifically, *Allium sp.* could be used as medicine because of its various active compound with proven medicinal effect, based on previous study [10, 11, 12]. Flavonoids are proven to be an anti-cancer agent by inhibiting the activated receptor, thus, poses higher chance as anti AML drug candidate.

FLT3 is a class III protein tyrosine kinase receptor. This protein is encoded by a gene located on chromosome 13q12. This receptor is highly expressed on hematopoietic cells. These receptors are also found in other organs such as the brain, placenta, and gonads. These receptors play an important role in cell growth and hematopoiesis. The good reproducibility of stem and myeloid cells is influenced by the FLT3 receptor. In addition, this receptor is also key to the differentiation and survival process of these cells. Because of the role of the FLT3 receptor, gene mutations in this receptor can lead to AML cancer [45, 46, 47].

Gene mutations in the FLT3 receptor experienced by individuals with AML disease account for 30% of the total cases. It is known that the overexpression of the FLT3 receptor in AML cases is the most experienced compared to other types of cancer [46,48]. There are two types of FLT3 mutations that occur in the majority of survivors, namely internal tandem duplications (ITD) and activation loop mutations. ITD mutations are mutations that occur due to partial duplication of fragments in the juxta-membrane domain and coding region. This mutation occurs most frequently (15-35% of total cases). These mutations cause changes in receptor activity and lead to activation of signaling pathways such as STAT5, MAPK/ERK, and PI3K/Akt [47,48,49]. Meanwhile, activation loop mutations occur due to point mutations in the tyrosine kinase domain (TKD). The cause of most TKD mutations in FLT3 is the conversion of aspartic acid to tyrosine at codon 835 [47,48]. Thus, it can be said that the inhibition of the mutated FLT3 receptor can be used as a therapeutic treatment.

The mechanism of FLT3 inhibition is divided into two schemes: generation and type of FLT3 inhibitors. The first-generation targets not only FLT3, but other kinases as well. The inhibition of FLT3 is accomplished via blocking other kinase parallel pathways, which is not clearly stated. Second-generation FLT3 inhibitors are more promising because they bind to FLT3-specific ligands and have little off-target effect at clinically relevant doses. Type I FLT3 inhibitors bind to the receptor in the active conformation, thereby inhibiting the FLT3-TKD (tyrosine kinase domain) and FLT3-ITD (internal tandem duplication) mutant receptors. Type II inhibitors bind to the region adjacent to the ATP-binding pocket and only inhibit the receptor in the inactive conformation [50,51]. Based on these mechanisms, the usage of small molecules from bioactive compounds can be applicated.

In this research FLT3 poses as the target protein, while the flavonoids from *Allium* sp. as the ligand. *Allium* sp. have dozens of flavonoids, yet only some of them present in high percentage. The major representatives are quercetin, kaempferol, isorhamnetin, myricetin, fisetin, and morin. [12]. Though, Quercetin-3,4-*O*-diglucoside, and spiraeoside are the most dominant, constituting 80-85% of total flavonoids content in some species or cultivars [13]. Another important compound is Que-3-O-glucoside (Isoquercetin) and Que-3-O-rhamnoside (Quercitrin), found in almost all species of *Allium* and serving high anticancer potency [12]. Regardless of that, the screening result shown in the Table 1 was rather displeasing. All the important quercetin derivatives were not passed the drug-likeness screening since it violated the Lipinski Rule of 5 (LRO5), thus, only quercetin and kaempferol suitable for drug candidate.

Nevertheless, all listed compounds still be analyzed by molecular docking and dynamics and the final result shows promising analysis results. The protein-ligand interaction depicted at Table 2 show the expected result. The interaction between the ligand and its function as a drug with the target receptor is expected to last a long time. Bonding between drug compounds and receptors can be categorized into several types based on their polarity. Drugs with low polarity will interact with receptors through hydrogen and van der Waals bonds, while compounds with high polarity will interact ironically [52]. Based on previous research, it is known that hydrogen bonding and hydrophobic bonding can increase the stability of drug compounds at the target site. This stability affects the efficacy and value of binding affinity [53].

CONCLUSION

Ten flavonoids with the highest percentage of *Allium* sp. selected as ligands include quercetin, kaempferol, isorhamnetin, myricetin, fisetin, morin, isoquercetin, quercitrin, spiraeoside, and

quercetin-3,4-O-diglucoside. All selected ligands were tethered to the FLT3 receptor along with gilteritinib as a control. The results showed that quercetin, kaempferol, isoquercetin, quercitrin, spiraeoside, and quercetin-3,4-O-diglucoside have the possibility to inhibit FLT3 activity because they bind to the same binding pocket as gilteritinib. Meanwhile, isorhamnetin, myricetin, fisetin, and morin have different binding properties compared to gilteritinib as a control. Quercetin, kaempferol, Isoquercetin, Quercitrin, Spiraeoside, and Quercetin-3,4-O-diglucoside also showed high binding affinity, thus confirming its potential as an anti-AML compound, supported by the molecular dynamic result to pointed out the stability of the complex.

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