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Research Article

Molecular Mechanism of Inhibition of Cell Proliferation: An In Silico Study of the Active Compounds in *Curcuma longa* as an Anticancer

Selliana Maretha Wijaya Kusuma[1], Didik Huswo Utomo[2], R. Susanti[1]*
1) Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Jalan Taman Siswa, Kampus Sekaran, Gunungpati, Semarang, Central Java 50229, Indonesia
2) Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.
* Corresponding author, email: basanatha8@mail.unnes.ac.id

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ABSTRACT
Cancer is one of the death causes in the world. Many plants act as anticancer, one of them is *Curcuma longa*. The purpose of this study was to analyze the molecular mechanism of compounds in *Curcuma longa* as an anticancer using in silico. These research methods included exploration of the active compounds of *Curcuma longa* plants, prediction of their activity, human intestinal absorption test, test of Lipinski's rule of five, molecular docking, and interactions of receptor with compounds as well as signaling pathways. The results showed that *Curcuma longa* had 20 compounds that have the potential as an anticancer. As many as 5 of the 20 active compounds, namely α-curcumene, curcumenol, curcumin, curcumin II, and curcumin III had a value of Pa > 0.3 and HIA above 80%. The results of molecular docking of α-curcumene, curcumenol, curcumin, curcumin II, and curcumin III compounds with protein receptors of VEGFR-2, EGFR, and FGFR-1 showed ΔG_{bind} values of -5.0 to -7.5 kcal/mol. The compound in *Curcuma longa* that had the most effective activity as an anticancer was curcumin with a ΔG_{bind} value of -7.5 kcal/mol at the FGFR-1 receptor. Curcumin molecular mechanism as antiproliferative was revealed computationally through inhibition of the PI3K/AKT/mTOR pathway.

INTRODUCTION
Cancer is one of the leading causes of human death in the world, characterized by the presence of some cells that grow uncontrollably and spread to other parts of the body (American Cancer Society 2016). Understanding the molecular changes in cancer development is one of the key factors to prevent and treat cancer and underlies the development of anticancer drugs (Hamzehzadeh et al. 2018; Tomeh et al. 2019). Various studies were conducted to find new drugs that have the potential as anticancer, one of them is turmeric (*Curcuma longa* Linn.).

Curcuma longa Linn. belongs to the Zingiberaceae family, and is a native plant of Asia, especially in India, Indonesia, and China (Abdurrahman 2019). Turmeric phytochemical studies show that turmeric contains curcuminoids

and essential oils as its main components. Curcumin and its derivatives have great attention in the last decade because of their anticancer activity (Nagahama et al. 2016; Chao et al. 2018). Curcumin is safe to use in animals and humans because curcumin can still be accepted by the body though in very high doses. However, curcumin has low bioavailability and low water solubility (Anisa et al. 2020). The properties of curcumin and its derivatives in cancer treatment led to the tyrosine kinase signaling pathway because curcumin inhibits receptor tyrosine kinases (RTK). The changes in genetic and gene expression of tyrosine kinase are responsible for the loss of cell growth control and oncogenic properties that appear in cancer (Golonko et al. 2019).

The first member of the receptor tyrosine kinases (RTK) superfamily is the epidermal growth factor receptor (EGFR). Other RTK proteins in humans, namely vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR) are key to the activity of the PI3K/AKT/mTOR signaling pathway (Rahimi 2017; Rawluk & Waller 2018; Golonko et al. 2019; Astolfi et al. 2020), which regulates cell proliferation, survival, and differentiation (Papadimitrakopoulou 2012; Hamzehzadeh et al. 2018). This study aimed to analyze the molecular mechanism of compounds in *Curcuma longa* as an anticancer using in silico.

MATERIALS AND METHODS

Materials

The materials used in this study were ligands (compounds in *Curcuma longa*) obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and receptors obtained from the PDB database (Protein Data Bank) (https: //www.rcsb.org/).

Methods

This study was an exploratory descriptive study which included exploration of the active compound of *Curcuma longa*, its activity prediction, human intestinal absorption test, Lipinski's rule of five test, molecular docking, and interaction of receptor with compounds and signaling pathways.

Collection of active compounds in *Curcuma longa*

The active compound in *Curcuma longa* was collected from Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke from the Agricultural Research Service/USDA (Ezealisiji & Awucha 2020), which can be accessed via (https://Phytochem.nal.usda.gov/) using keywords of *Curcuma longa*. SMILES compounds collection was carried out using PubChem via (https://pubchem. ncbi.nlm.nih.gov/). SMILES is used to predict the bioactivity of a compound.

Prediction of compound activity in *Curcuma longa* using test of PASS (prediction of activity spectra for substances)

The PASS Online test in this study was used to predict the possibility of

compound activity in preventing, inhibiting, and killing the growth and spread of cancer cells based on activities of pharmacological, biological, and ADME (absorption, distribution, metabolism, and excretion). PASS Online is a computer-based program to predict the biological activity of a compound (Jamkhande et al. 2014). The PASS test was carried out online via http://www.pharmaexpert.ru/passonline/. The results of the PASS test for each compound showed Pa (Potential Activity) and Pi (Potential Inhibitory) values based on the activity similarity of a compound structure with the drug compound. The PASS test results were analyzed and grouped based on the Pa value.

Test of HIA (human intestinal absorption) and toxicity hazard

The HIA test was carried out to predict the absorption ability of *Curcuma longa* compounds on intestinal wall. This test was carried out online using admetSAR (Moon et al. 2017) via http://lmmd.ecust.edu.cn/admetsar2/ by entering the SMILES compounds in the search column and running until the data was generated. The results of the HIA test were analyzed based on the percent value (%), with values of high (70-100%), medium (20-70%), and low (0-20%). Toxicity hazard (when administrated orally) was predicted using Toxtree. It was categorized into three classes, that are low (class I) which indicates efficient mode of metabolism, intermediate (class II) which possess less innocuous than class I, and high (class III) which indicates significant toxicity or had reactive functional group so the dose was crucial for oral use.

Test of Lipinski's rule of five

Test of Lipinski's rule of five is to determine the ability of compounds to penetrate cell membranes and reach target receptors (Jadhav et al. 2015). According to this law, a drug compound must comply with two or more absolute values of Lipinski's rules consisting of (1) a molecular weight is less than 500 g/mol, (2) a log P-value is less than 5, (3) a value of Hydrogen Bond Donors (HBD) is not more than 5, (4) the value of the Hydrogen Bond Acceptor (HBA) is not more than 10, and (5) the value of the Molar refractivity should be between 40-130 (Syahputra et al. 2014). Test Lipinski's rule of five was carried out through admetSAR on the http://lmmd.ecust.edu.cn/admetsar2/. The results of Lipinski's rule of five were analyzed by grouping the test ligand compounds that fulfilled 4 or 5 criteria of the rule of five.

Molecular docking and visualization

The structure of *Curcuma longa* compound and anticancer drugs downloaded through PubChem (https://pubchem.ncbi.nlm.nih.gov/) in 3D, saved in SDF format (*sdf) (Dallakyan & Olson 2014). The use of anticancer drugs of sunitinib, lapatinib, and gefitinib were selected based on the ability of the drug mechanism to act on a protein receptor through the drug bank (https://go.drugbank.com/). The receptor structure was downloaded through the protein data bank (https://www.rcsb.org/). The downloaded

receptor structure data was prepared using the discovery studio visualizer application. Furthermore, the removal of water molecules and ligands on protein macromolecules that were not needed was carried out. The structure formed was saved in .pdb format, which then can be used in the docking process.

Molecular docking between ligands (*sdf) and protein macromolecules (*pdb) was performed using Pyrx 0.8 software and analyzed by AutoDock Vina which functions as an anchor. Vina AutoDock is found on Pyrx which is included in the Vina Wizard section (Dallakyan & Olson 2014). In addition to the active compounds and target proteins, molecular docking was also carried out on the drug control compounds to determine the similarity of interactions between the test compounds and the control compounds. The molecular docking results were binding affinity scores, or bond energy values (ΔG_{bind}) and the interaction results were visualized into 2D and 3D structures using the Discovery Studio Visualizer. The results of molecular docking were analyzed by grouping the ligands based on the value of the bond energy (ΔG_{bind}), the type of bond formed, and the analysis of signaling mechanism.

Receptor interaction with compounds and signaling pathways

The interaction of three receptors (VEGFR-2, EGFR, and FGFR-1) with *Curcuma longa* compounds was evaluated to know the relationship between receptors and compounds in analyzing signaling pathways or biological pathways in cancer by their proteins. This interaction can be analyzed using STRING on page https://string-db.org/ (Franceschini et al. 2013). The next step was to analyze the cancer inhibition signaling pathway using the KEGG pathway.

RESULTS AND DISCUSSION

Predictions of active compounds from *Curcuma longa* based on Dr. Duke's phytochemical and ethnobotanical databases obtained 267 compounds with 658 activities. From 267 compounds, 20 compounds that have activities of anticarcinogenic, anticancer, and anti-tumor were found (Table 1).

This database (Dr. Duke's phytochemical and ethnobotanical databases) is often used to predict the activity of a test compound. Maduabuchi and Awucha (2020) used this database to predict the activity of *Pterocarpus mildbraedii* compounds as medicine for malaria and digestive disorders. Anand and Gokulakhrisna (2014) used Dr. Duke's databases in a study related to *Hybanthus enneaspermus* plant activity, especially the characteristics of bioactive compounds in ethanol extracts.

A total of 20 phytochemical compounds of *Curcuma longa* were obtained, based on the results of PASS Online screening, 5 compounds with anticarcinogenic activity were obtained, with a Pa value more than 0.3, namely curcumene, curcumenol, curcumin, curcumin II, and curcumin III (Table 2). According to Filimonov et al. (2014), the PASS test performs an analysis based on the relationship of the compound structure and its biological activi-

ty or SAR (Structure Activity Relationship).

Table 1. Collection of *Curcuma longa* compounds as anticancer.

No	Compound	Molecular Formula
1	Alpha-curcumene (Curcumene)	$C_{15}H_{22}$
2	Alpha-terpineol	$C_{10}H_{18}O$
3	Alpha-tocopherol	$C_{29}H_{50}O_2$
4	Ar- tumerone	$C_{15}H_{20}O$
5	Ascorbic acid	$C_6H_8O_6$
6	Beta carotene	$C_{40}H_{56}$
7	Beta sitosterol	$C_{29}H_{50}O$
8	Beta turmerone	$C_{15}H_{22}O$
9	Bis-desthoxycurcumin (Curcumin III)	$C_{19}H_{16}O_4$
10	Caryophyllene	$C_{15}H_{24}$
11	Curcumenol	$C_{15}H_{22}O_2$
12	Curcumin	$C_{21}H_{20}O_6$
13	Curcuminoid	$C_{21}H_{18}N_2O_6$
14	Curcumenol	$C_{15}H_{22}O_2$
15	Curcumol	$C_{15}H_{24}O_2$
16	Curdione	$C_{15}H_{24}O_2$
17	Demethoxycurcumin (Curcumin II)	$C_{20}H_{18}O_5$
18	Limonene	$C_{10}H_{16}$
19	Quercetin	$C_{15}H_{10}O_7$
20	Tetrahydrocurcumin	$C_{21}H_{24}O_6$

Curcumin, curcumin II, and curcumin III had anticancer activity (anticarcinogenic) in moderate criteria ($0.5 < Pa < 0.7$). Pa value indicated that the compound has probability to be active and in mid-range score is a good candidate for drug discovery. While curcumene and curcumenol compounds had low anticancer activity ($Pa < 0.5$) prediction. The greater the Pa value, the more possibility of the compound to block receptors in laboratory experiments. Nevertheless, compounds with low Pa value are not certain to have low activity, because there have not been many studies on these compounds (Pramely & Raj 2012; Filimonov et al. 2014; Ivanov et al. 2018).

Table 2. PASS online prediction results.

Compound	Value	
	Pa	Pi
α-Curcumene	0.357	0.039
Curcumenol	0.454	0.024
Curcumin	0.611	0.012
Curcumin II	0.645	0.011
Curcumin III	0.555	0.015

Comparative analysis of the Pa and Pi values of the five compounds in *Curcuma longa* showed that all of them had Pa value above 0.3 (Table 2). Only compounds with Pa value > 0.3 were categorized as good activity (Pramely & Raj 2012; Druzhilovskiy et al. 2016).

Prediction of compound absorption with parameters of HIA

Based on the results of the HIA (Human Intestinal Absorption) prediction,

the HIA values for α-curcumene, curcumenol, curcumin, curcumin II, curcumin III, sunitinib, gefitinib, and lapanitib were more than 84% (Table 3). The value obtained showed that anticancer drugs and these compounds can be well absorbed in the intestines because they had a value of more than 70%. According to Nerkar et al. (2012), if the predicted absorption of a compound is more than 70%, it can be stated that the intestines have a high ability to absorb these compounds and can reach their target receptors. The result of toxicity hazard also suggests that the compound has reactive functional group when orally administrated. For medical application the effective dose is crucial to be considered. All active compounds from *Curcuma longa* could be used as a treatment by considering all pharmacokinetics properties. Compounds in *Curcuma longa* are predicted to have the same speed as anticancer drugs to reach the target of cancer cell receptors in the body, thus blocking the receptors from binding and inhibiting the growth of cancer cells (Lazzeroni et al. 2012).

Table 3. Result of HIA prediction and toxicity hazard for oral use.

		Toxic Hazard		
Compounds	HIA (%)	Low (Class I)	Intermediate (Class I)	High (Class I)
α-Curcumene	98.87	○	-	-
Curcumenol	84.59	-	-	○
Curcumin	97.70	-	-	○
Curcumin II	97.70	-	-	○
Curcumin III	97.37	-	-	○
Sunitinib	97.60	-	-	○
Lapatinib	98.14	-	-	○
Gefitinib	99.37	-	-	○

Note: ○= categorized; - = Not categorized.

Prediction of compound potential using parameters of Lipinski's rule of five

When a compound is able to be absorbed in intestinal wall and enters the blood circulation properly, it will be distributed throughout the body and penetrate the cell membrane to reach its target receptor. Therefore, it is important to pay attention to factors related to pharmacology, because drug interactions will not occur if the compound can't reach its target (Jadhav et al. 2015).

Lipinski's prediction results showed that 5 compounds of *Curcuma longa* and 2 compounds of anticancer drugs (sunitinib and gefitinib) met all the criteria of the rule of five. Lapatinib did not meet the 3 criteria, namely a molecular mass of more than 500 Da, a LogP of more than 5, and a molar refractivity of more than 130 (Table 4). However, lapatinib still complied with the Lipinski's rule of five, because it still had 2 criteria (Jadhav et al. 2015).

Table 4. Results of Lipinski's rule of five test for ligand compounds.

Ligand	Molecular Mass (Da)	H- Donor	H- Acceptor	LogP	Molar Refractivity
α-Curcumene	202	0	0	4.84	69.55
Curcumenol	234	1	2	3.18	69.25
Curcumin	368	2	6	3.37	102.80
Curcumin II	338	2	5	3.14	96.31
Curcumin III	308	2	4	3.35	89.82
Sunitinib	398	3	3	3.33	116.31
Lapatinib	581 *	2	8	6.14 *	153.88 *
Gefitinib	446	1	7	4.28	121.66

*Value does not meet requirements of the rule of five.

According to Syahputra et al. (2014), compounds with molecular mass of more than 500 will be difficult to penetrate cell membrane, because they do not diffuse into cell. Conversely, compounds with small molecular mass can enter the cell through the diffusion process. Almost all of the tested compounds also had values of H-donor and H-acceptor less than 5, so these compounds can penetrate cell membranes. Too large value of H-donor and H-acceptor will slow down the compound to reach the target. Likewise, with the logP value, if the value is too large, the compound will be difficult to pass through the cell membrane and tend to have a high level of toxicity. The greater the logP value, the more hydrophobic the molecule and more easily retained in the lipid bilayer of the cell membrane. It will cause the compound to be widely distributed, thereby reducing bond selectivity of compounds to the target (Kilo et al. 2019; Weni et al. 2020).

Molecular docking of active compound from *Curcuma longa*

The result of the molecule binding to the target is binding affinity (ΔG_{bind}) and the interaction of ligand with protein receptor. The results of the ligand-receptor interaction are based on ΔG_{bind} value because each binding of the ligand to the protein macromolecule (receptor) produces a ligand conformational based on ΔG_{bind} rank. The smaller the ΔG_{bind} value, the more stable the ligand binding to the receptor. ΔG_{bind} is energy required by ligand when it interacts or binds to the receptor binding site.

Table 5. Value of ΔG_{bind} (kcal/mol) docking of 7 compounds with 3 receptors.

Compound (Ligand)	Receptor		
	VEGFR-2	EGFR	FGFR-1
α-Curcumene	-5.8	-6.0	-6.8
Curcumenol	-6.9	-6.7	-7.2
Curcumin	-7.3	-7.1	-7.5
Curcumin II	-7.2	-7.3	-7.0
Curcumin III	-7.0	-7.2	-6.8
Sunitinib	-6.9	-7.6	-7.0
Lapatinib	-7.2	-7.8	-8.5
Gefitinib	-7.2	-7.4	-6.9

The value of ΔG_{bind} in Table 5 showed that curcumin could form a complex more efficiently than other compounds because it had the most

negative ΔG_{bind} (binding affinity) value. While in anticancer drugs, interaction of the FGFR-1 receptor with lapatinib had the highest ΔG_{bind} value of -8.5 kcal/mol. Interaction between ligands and macromolecular residues on receptor can be seen based on visualization using discovery studio visualizer software. The interactions that occur can be in the form of hydrogen, hydrophobic, and electrostatic bonds (Arwansyah et al. 2014). Affinity determination based on the negative or lowest ΔG_{bind} value is preferred, because it relates to the amount of hydrogen or various types of residues that interact with ligand. The difference in ΔG_{bind} value in each compound is influenced by the bonds formed. Role of hydrogen bond determines the value of resulting ΔG_{bind} rather than hydrophobic bond. However, hydrophobic bond plays an important role in determining the stability of ligand to receptor (Arwansyah et al. 2014).

Based on Table 6, it can be seen that the compound of curcumin-FGFR-1 had fewer hydrogen bonds (3) than curcumin II (8), but the ΔG_{bind} value of curcumin-FGFR-1 (-7.5) was higher than curcumin II-FGFR-1 (-7.0). Likewise, the lapatinib-FGFR-1 interaction had fewer hydrogen bonds (1) than gefitinib (12) and sunitinib (3), but the ΔG_{bind} of lapatinib-FGFR-1 was higher (-8.5) than gefitinib (-6.9) and sunitinib (-7.0). The similarity of the amino acid residues that interacted between ligand compounds and control drug showed that the ligand had potential as a substitute for the control drug. The molecular docking results for *Curcuma longa* compounds which have same amino acid residues with control cancer drugs were summarized in Table 7.

Interaction similarity of amino acid residues of ligand with control drug indicates that ligand is able to inhibit activity of target protein and has the potential to substitute control drug (Arwansyah et al. 2014; Chamata et al. 2020). Active compounds that have strong binding to target protein receptors are compounds that have same hydrogen bonds and amino acid residues as control drugs (Bintari 2018). From the docking results, it was found that not all ligand compounds had same amino acid residue as control drug. Curcumin compounds had same amino acid residues as the three drugs, both in the interaction with VEGFR-2, EGFR, and FGFR-1. It showed that curcumin bound well to FGFR1 in the binding pocket and can be a substitute for

Table 6. Number of hydrogen and hydrophobic bonds in the interaction of *Curcuma longa* compounds (ligands) with receptors.

Compound (Ligand)	VEGFR-2		EGFR		FGFR-1	
	Hydrogen Bond	Hydrophobic Bond	Hydrogen Bond	Hydrophobic Bond	Hydrogen Bond	Hydrophobic Bond
α-Curcumene	-	12	-	10	-	14
Curcumenol	-	12	-	11	-	15
Curcumin	4	7	3	14	3	12
Curcumin II	3	11	2	15	8	12
Curcumin III	1	12	1	15	5	11
Sunitiib	1	8	1	15	3	12
Lapatinnib	2	15	4	12	1	17
Gefitiib	6	11	5	13	12	12

cancer drugs, according to the results of in vivo studies (Figure 1) (Puteri 2020). According to Devassy et al. (2015), curcumin had been shown to have many positive effects on suppressing transformation, angiogenesis, metastasis, and suppressing cancer cell growth through cell proliferation. It is stated that curcumin has effect of preventing various types of cancer, such as prostate, pancreatic, lung, colon, and breast cancer. The three anticancer drugs are cancer inhibitors (data from the DrugBank website). Sunitinib inhibits cellular signaling by targeting multiple RTK, lapatinib inhibits RTK, and gefitinib inhibits the intracellular phosphorylation of many tyrosine kinases associated with cell transmembrane surface receptors.

Table 7. Amount of similarity of amino acid residues between *Curcuma longa* compounds and anticancer drugs.

Ligand	Receptor		
	VEGFR-2	EGFR	FGFR-1
α-Curcumene - Sunitinib	0	0	0
Curcumenol - Sunitinib	0	14	0
Curcumin - Suntinib	0	10	3
Curcumin II - Sunitinib	5	12	1
Curcumin III - Sunitinib	0	10	1
α-Curcumene - Lapatinib	2	0	0
Curcumenol - Lapatinib	0	8	1
Curcumin - Lapatinib	1	9	2
Curcumin II - Lapatinib	6	9	3
Curcumin III - Lapatinib	0	7	8
α-Curcumene - Gefitinib	12	0	0
Curcummenol - Gefitinib	0	7	1
Curcumin - Gefitinib	9	9	4
Curcumin II - Gefitinib	2	13	1
Curcumin III - Gefitinib	0	10	3

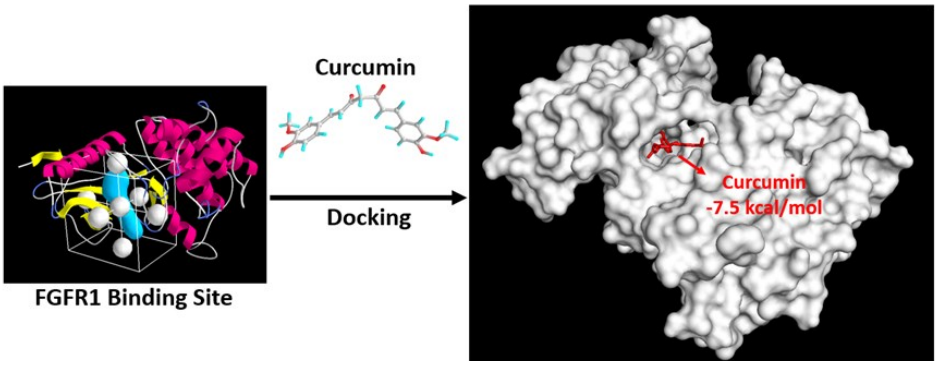

Figure 1. Binding pocket detection and the molecular interaction between curcumin and FGFR1 with binding energy -7.5 kcal/mol.

The number of similarities of amino acid residues possessed by each ligand compound does not affect the ΔG_{bind} value. There were many similarities in amino acid residues in the three control drugs to α-curcumenol, curcumin, curcumin II, and curcumin III, but curcumin had the highest ΔG_{bind} value (-7.5 kcal/mol). Study results by Arfi et al. (2020) showed that cyclocurcumin ligand had a stronger potential to bind to its target protein than

mebendazole ligand, although cyclocurcumin ligand had fewer amino acid residues than mebendazole ligand.

PI3K/AKT signaling pathway analysis

The signaling pathway mechanism was analyzed using STRING to determine the signaling interactions of proteins to form interaction networks. Proteins that affect signaling were marked in red with various line colors. Blue lines show interactions based on an accurate database, purple lines show interactions based on experimental research, green lines show interactions based on gene-environment predictions, red lines show cell fusion, dark blue lines show gene-occurrence interactions, yellow lines show text mining, black lines show co-expression, and gray lines show protein homologs (Franceschini et al. 2013). Pathway analysis used KEGG pathway contained in STRING by entering the three receptors in the column presented and selecting PI3K/AKT pathway on the KEGG list. STRING results were shown in Figure 2.

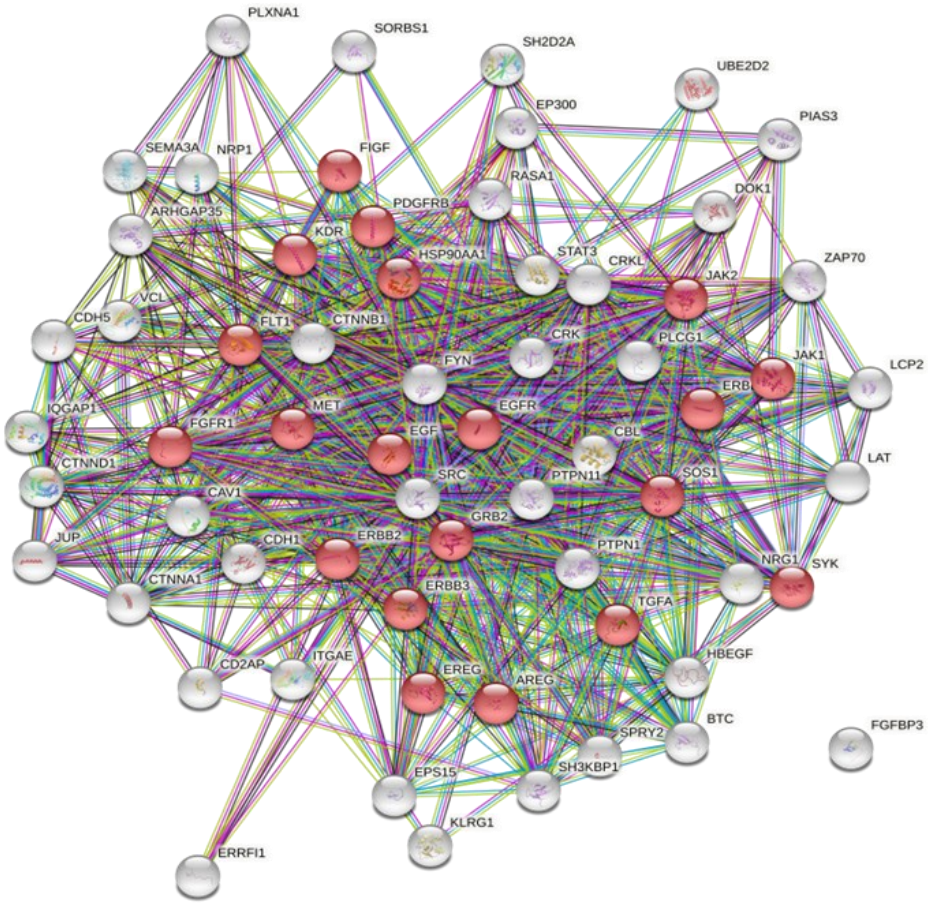

Figure 2. Results of STRING analysis of on KEGG pathway.

The results of the protein interaction network showed that many proteins contribute to the same function. Several proteins that contribute to the PI3K/AKT pathway include KDR (VEGFR-2), EGFR, and FGFR-1. Curcumin compounds in *Curcuma longa* can inhibit angiogenesis, induce apoptosis, and suppress cancer cell proliferation. Inhibition mechanism of tumorigenesis and anticarcinogenic in curcumin can occur through various levels of

signaling pathways, such as NF-Kb, PI3K/AKT, mTOR, Ras/Raf/Erk, STAT, etc. (Abdurrahman 2019; Cas & Ghidoni 2019). Mechanism of curcumin action occurs both intrinsically and extrinsically (Abdurrahman 2019).

Curcumin can inhibit cell proliferation, differentiation, migration, and transcription, and also increase apoptotic death by inhibiting its signaling pathways. Apoptosis induction, growth inhibition, and differentiation enhancement by curcumin occur through PI3K/AKT signaling pathway (Figure 3). In PI3K/AKT signaling pathway, curcumin reduces malignant potential of cancer cells by inhibiting cell proliferation (Hamzehzadeh et al. 2018).

Phosphatidylinositol-3-kinase (PI3K) signaling is a pathway that plays an important role in aspects of cell growth, cell cycle, apoptosis, and cell proliferation in cancer development. Therefore, this signaling pathway is closely related to tumor development and can be an important anticancer target (Shi et al. 2019).

RTK (receptor tyrosine kinase) is a cell surface receptor for many growth factors, hormones, and cytokinins (Mele & Johnson 2019). RTK has phosphotyrosine kinase activity which is attached to plasma membrane but in inactive condition (Haddadi et al. 2018). RTK activation occurs due to the binding of ligands (extracellular molecules) nor growth factors and receptor-associated proteins (Insulin Receptor Substrate-1; IRS1). When ligand binds to RTK, it activates an intracellular tyrosine kinase domain that results in phosphorylation of tyrosine receptors on cell surface and attracts PI3K (phosphatidylinositol 3-kinase) to plasma membrane. The p85 regulatory subunit of PI3K binds directly to the tyrosine receptor. The binding of p58 results in activation of the p110 catalytic subunit in plasma membrane, then p110 catalyzes the phosphorylation of PIP2 (phosphatidylinositol-4,5-bisphosphate) to PIP3. PIP3 regulates many cellular processes such as cell proliferation, angiogenesis, growth, survival, and motility (Papadimitrakopoulou 2012).

RTK activation further activates the PI3K/Akt/mTOR pathway, a central pathway for cancer growth, survival, and motility, making this pathway a target for cancer research and therapy (Cidado & Park 2012). PIP3 contributes to the recruitment and activation of various downstream signaling targets, including Akt (protein kinase B). PIP3 localizes Akt to plasma membrane and it is activated through phosphorylation of phosphoinositide-dependent kinase (PDK1). After phosphorylated, Akt escapes from plasma membrane and activates targets that promote the increase of cell growth, metabolism, survival, and cell proliferation in nucleus and cytoplasm. Akt which is phosphorylated by PIP3 second messenger will activate mTOR (mammal target of rapamycin), mTOR2 will phosphorylate Akt for the second time after PIP3 is fully activated. mTOR acts as a regulator of cell proliferation and growth located downstream and upstream of Akt (Papadimitrakopoulou 2012).

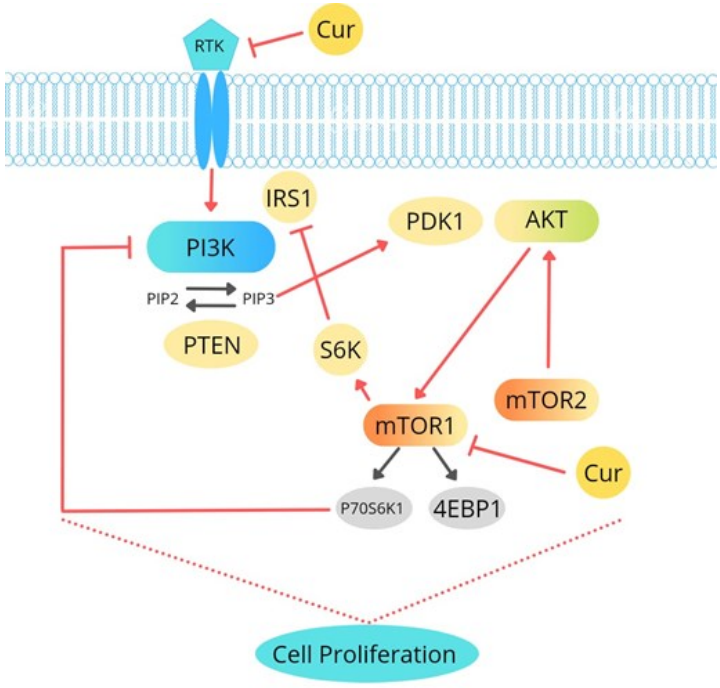

Figure 3. Targeting anti-cancer signals by curcumin.

Curcumin inhibits Akt phosphorylation in cancer cells thereby inhibiting mTOR signaling. Akt Inhibition is due to dephosphorylation of PIP3 to PIP2 which is inactive by PTEN or other inhibition by mTOR. Curcumin reduces cancer cell proliferation by reducing the expression of Raptor and Rictor components. mTORC1 has a significant role in controlling cell proliferation and the downstream pathway is 70 kDa ribosomal protein S6 kinase 1 (p70S6K1) and eIF4E 1 (4EBP1) binding protein which can provide negative feedback on Akt pathway. mTORC1 signaling is regulated by Akt through TSC1/TSC2 complex, which can induce GTPase and Rheb activity. Curcumin directly suppressed Akt and mTORC1 so the inhibition of downstream pathway like phosphorylation of p70S6K1 and 4EBP1 can be achieved. These downstream targets are involved in cell proliferation and growth (Tamaddoni et al. 2020).

CONCLUSION

Curcuma longa is well-known as anticancer and curcumin as one of metabolites could be concluded that had the most promising anticancer activity by interaction with FGFR-1 receptor protein. Curcumin molecular mechanism as antiproliferative was revealed computationally through by inhibiting the PI3K/AKT/mTOR pathway. The anti-cancer activity of *Curcuma longa* compounds needs to be tested further by in vivo and in vitro to find out more about the inhibitory activity of *Curcuma longa* compounds as anticancer.

AUTHORS CONTRIBUTION

This research was conducted with the cooperation of all the authors, with the following details: conceptual and design research by RS and DHU; data collection by SMWK; data validation and accuracy by RS; data visualization by

DHU; writing original draft manuscript by SMWK; review of draft manuscripts by DHU and RS; supervision of all activities by RS.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

Abdurrahman, N., 2019. Kurkumin pada Curcuma longa sebagai Tatalaksana Alternatif Kanker (Curcumin in *Curcuma longa* as an Alternative Cancer Treatment). *Journal Agromedicine*, 6(2), pp.410-415.

American Cancer Society, 2016. Cancer Fact and Figure. Atlanta: American Cancer Society Inc.

Anand, T. & Gokulakrishnan, K., 2014. GC-MS Analysis and Anti-Microbal Activity of Bioactive Components of *Hybanthus enneaspermus*. *International Journal of Pharmacy and Pharmateucal Science*, 2(3), pp.646-650.

Anisa, D.N., Anwar, C. & Afriyani, H., 2020. Sintesis Senyawa Analog Kurkumin Berbahan Dasar Veratraldehida Dengan Metode Ultrasound. *Analit: Analytical and Environmental Chemistry*, 5(01), pp.74-81. doi: 10.23960%2Faec.v5i1.2020.p74-81

Arfi, A.S., Lestari, R.D. & Damayanti, D.S., 2020. Studi In Silico Senyawa Aktif Rimpang Kunyit (*Curcuma domestica*) terhadap Penghambatan Acetylcholinesterase, Microtubulin (Beta tubulin), dan Aktivasi Calcium channel sebagai Terapi Antelmintik. (In Silico Study: Active Compound of Turmeric Rhizome (Curcuma domestica) Towards Acetylcholinesterase and Microtubulin (Beta Tubulin) Inhibition, and Calcium Channel Activation as an Anthelmintic Therapy). *Jurnal Kedokteran Komunitas*, 8(2), pp.36-47.

Arwansyah, A., Ambarsari, L. & Sumaryada, T.I., 2014. Simulasi Docking Senyawa Kurkumin dan Analognya Sebagai Inhibitor Reseptor Androgen pada Kanker Prostat. *Current Biochemistry*, 1(1), pp.11-19. doi: 10.29244/cb.1.1.11-19

Astolfi, A. et al., 2020. The Emerging Role of the FGF/FGFR Pathway in Gastrointestinal Stromal Tumor. *International Journal of Molecular Science*, 27(3313), pp.1-14. doi: 10.3390/ijms21093313

Bintari, Y.R., 2018. Studi In Silico Potensi Ekstrak Lipida *Tetraselmis chuii* sebagai Antioksidan. *Jurnal Ketahanan Pangan*, 2(1), pp.76-81.

Cas, M.D. & Ghidoni, R. 2019. Dietary Curcumin: Correlation Between Bioavailability and Health Potential. *Nutrient*s, 11(2147), pp.1-14. doi: 10.3390/nu11092147

Chamata, Y., Watson, K.A. & Jauregi, P., 2020. Whey-Derived Peptides Interactions with Ace by Molecular Docking as A Potential Predictive Tool of Natural Ace Inhibitors. *International Journal of Molecular Sciences*, 21(3), pp.1-13. doi: 10.3390/ijms21030864

Chao, I.C. et al., 2018. Simultaneous Quantification of Three Curcuminoids and Three Volatile Components of *Curcuma longa* Using Pressurized Liquid Extraction and High-Performance Liquid Chromatography. *Molecules*, 23(7), pp.1568. doi: 10.3390/molecules23071568

Cidado, J. & Park, B.H., 2012. Targeting the PI3K/Akt/mTOR Pathway for Breast Cancer Therapy. *Journal Mammary Gland Biology and Neoplasia*, 17 (3-4), pp.205-216. doi: 10.1007/s10911-012-9264-2

Dallakyan, S. & Olson, A.J., 2014. Small-Molecule Library Screening by Docking with PyRx. *Methods in Molecular Biology*, 1263, pp.243-250. doi: 10.1007/978-1-4939-2269-7_19

Devassy, J., Nwachukwu, I. & Jones, P., 2015. Curcumin and Cancer: Barriers to Obtaining a Health Claim. *Nutrition Reviews*, 73(3), pp.155-165. doi: 10.1093/nutrit/nuu064

Druzhilovskiy, D. S. et al., 2016. Online Resources for the Prediction of Biological Activity of Organic Compounds. *Russian Chemical Bulletin*, 65(2), pp.384-393. doi: 10.1007/s11172-016-1310-6

Ezealisiji, K.M. & Awucha, N.E., 2020. Phytochemistry and GC-MS analysis of methanolic leaf extract of *Newbouldia leavis* (Bignonacea). *Journal of Pharmacognosy and Phytochemistry*, 9(2), pp.1998-2004.

Filimonov, D.A. et al., 2014. Prediction of the Biological Activity Spectra of Organic Compounds Using the PASS Online Web Resource. *Chemistry of Heterocyclic Compounds*, 50(3), pp.444-457. doi: 10.1007/s10593-014-1496-1

Franceschini, A. et al., 2013. STRING v9.1: Protein-Protein Interaction Networks, With Increased Coverage and Integration. *Nucleic Acids Research*, 41(1), pp.808-815. doi: 10.1093/nar/gks1094

Golonko, A. et al., 2019. Curcumin as Tyrosine Kinase Inhibitor in Cancer Treatment. *European Journal of Medicinal Chemistry*, 181(111512). pp.1-25. doi: 10.1016/j.ejmech.2019.07.015

Haddadi, N. et al., 2018. PTEN/PTENP1: 'Regulating the Regulator of RTK -Dependent PI3K/Akt Signalling', New Targets for Cancer Therapy. *Molecular Cancer*, 17(37), pp.1-14. doi: 10.1186/s12943-018-0803-3

Hamzehzadeh, L. et al., 2018. The Versatile Role of Curcumin in Cancer Prevention and Treatment: A Focus on PI3K/AKT Pathway. *Journal of Cellular Physiology*, 233(10), pp.6530-6537. doi: 10.1002/jcp.26620.

Ivanov, S.M. et al., 2018. ADVERPred-Web Service for Prediction of Adverse Effects of Drugs. *Journal of Chemical Information and Modeling*, 58(1), pp.8-11. doi: 10.1021/acs.jcim.7b00568.

Jadhav, P.B., Yadav, A.R. & Gore, M.G., 2015. Concept of Drug Likeness in Pharmaceutical Research. *International Journal of Pharma and Bio Science*, 6 (4), pp.142-154.

Jamkhande, P. G. et al., 2014. Antioxidant, Antimicrobial Activity and In Silico PASS Prediction of *Annona reticulata* Linn. Root Extract. *Beni-Suef University Journal of Basic and Applied Sciences*, 3(2), pp.140-148. doi: 10.1016/j.bjbas.2014.05.008

Kilo, A.L. et al., 2019. Studi Potensi Pirazolin Tersubstitusi 1-N dari Tiosemikarbazon sebagai Agen Antiamuba Melalui Uji in Silico (Study of Potential of 1-N-Substituted Pyrazoline Analogues of Thiosemicarbazones as Antiamoebic Agent using In Silico Screening). *Indonesia Journal of Chemical Research*, 7(1), pp.9-16. doi: 10.30598//ijcr.2019.7-akr

Lazzeroni, M. et al., 2012. Oral low dose and topical tamoxifen for breast cancer prevention: modern approaches for an old drug. *Breast Cancer Research*, 14(5), 1–11. doi: 10.1186/bcr3233

Maduabuchi, E.K. & Awucha, N.E., 2020. Phytochemistry and GC-MS Analysis of Methanolic Leaf Extract of *Newbouldia laevis* (Bignonacea). *Journal of Pharmacognosy and Phytochemistry*, 9(2), pp. 1998-2004.

Mele, S. & Johnson, T.K., 2019. Receptor Tyrosine Kinases in Development: Insights from Drosophila. *International Journal of Molecular Sciences*, 21 (188), pp.1-21. doi: 10.3390/ijms21010188

Moon, A. et al., 2017. Insilico Prediction of Toxicity of Ligands Utilizing Admetsar. *International Journal of Pharma and Bio Sciences*, 8(3), pp.674-677. doi: 10.22376/ijpbs.2017.8.3.b674-677

Nagahama, K. et al., 2016. Discovery of A New Function of Curcumin Which Enhances Its Anticancer Therapeutic Potency. *Scientific Reports*, 6(30962), pp.1-14. doi: 10.1038/srep30962

Nerkar, A.G. et al., 2012. In Silico Screening, Synthesis and Pharmacological Evaluation of Novel Quinazolinones as NMDA Receptor Inhibitors for Anticonvulsant Activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3), pp.449-453.

Papadimitrakopoulou, V., 2012. Development of PI3K/AKT/mTOR Pathway Inhibitors and Their Application in Personalized Therapy for Non -Small-Cell Lung Cancer. *Journal of Thorac Oncology*, 7(8), pp.1315-1326. doi: 10.1097/JTO.0b013e31825493eb

Pramely, R. & Raj, T.L.S., 2012. Prediction of Biological Activity Spectra of A Few Phytoconstituents of *Azadirachta indicia* A. Juss. *Journal of Biochemical Technology*, 3(4), pp.375-379.

Puteri, F.D., 2020. Efek Kurkumin Pada Kunyit (*Curcuma longa*) Sebagai Pengobatan Kanker Lambung (Effect of Curcumin on Turmeric (*Curcuma longa*) as a Tretment for Stomach Cancer). *Jurnal Ilmiah Kesehatan Sandi Husada*, 9(2), pp.860-864. doi: 10.35816/jiskh.v10i2.426

Rahimi, N., 2017. Defenders and Challengers of Endothelial Barrier Function. *Frontiers in Immunology*, 8(1847), pp.1-10. doi: 10.3389/ fimmu.2017.01847

Rawluk, J. & Waller, C.F., 2018. Gefitinib. *Recent Results Cancer Research*, 211, pp.235-246. doi: 10.1007/978-3-319-91442-8_16

Shi, X. et al., 2019. Research Progress on the PI3K/AKT Signaling Pathway in Gynecological Cancer (Review). *Molecular Medicine Reports*, 19(6), pp.4529-4535. doi: 10.3892/mmr.2019.10121

Syahputra, G, Ambarsari, L. & Sumaryada, T., 2014. Simulasi docking kurkumin enol, bisdemetoksikurkumin dan analognya sebagai inhibitor enzim12-lipoksigenase. *Biofisika*, 10(1), pp.55-67.

Tamaddoni, A. et al., 2020. The Anticancer Effects of Curcumin via Targeting the Mammalian Target of Rapamycin Complex 1 (mTORC1) Signaling Pathway. *Pharmacological Research*, 156(104798), pp.1-11. doi: 10.1016/j.phrs.2020.104798

Tomeh, M.A., Hadianamrei, R. & Zhao, X., 2019. A Review of Curcumin and Its Derivatives as Anticancer Agents. *International Journal of Molecular Sciences*, 20(1033), pp.1-26. doi: 10.3390/ijms20051033

Weni, M., Safithri, M. & Seno, D.S.H., 2020. Molecular Docking of Active Compounds Piper crocatum on The Alpha-Glucosidase Enzyme as Anticiabetic. *Indonesian Journal of Pharmaceutical Science and Thechnology*, 7 (2), pp.64-72. doi: 10.24198/ijpst.v7i2.21120