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Natural Compounds Derived from *Camellia sinensis* as Therapeutic Agent to Treat Non-Small Cell Lung Carcinoma (NSCLC): A Molecular Docking Study

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Abstract

Cancer is one of the biggest health problems with lung cancer as the first rank in the number of new cases and deaths. Non-small cell lung carcinoma (NSCLC) is a type of lung cancer which accounts for about 85% cases. Previous research identified the role of epidermal growth factor receptor (EGFR) as the most suitable target to treat NSCLC. This study used a molecular docking technique to identify the potential compounds derived from *Camellia sinensis* (green tea) leaves as therapeutic agent to treat NSCLC. We tested 12 compounds in green tea leaves along with gefitinib as a comparative drug. Docking was carried out on EGFR as receptor target by Autodock Tools and Autodock Vina. Molecular interactions were visualized by Discovery Studio v16. All compounds met the criteria as drugs based on Lipinski's solubility test and were safe to use based on toxicity test with AdmetSAR. Docking results showed that all compounds had affinity to EGFR receptor. Catechin and myricetin had the same energy bonds as gefitinib which were -7,9 kcal/mol, while theaflavin gallate, theaflavin digallate, epicatechin gallate, epigallocatechin-3-gallate, catechin gallate, thearubigin, quercetin, and kaempferol were proven to have the strongest binding energy compared to gefitinib which were -10.6, -9.8, -8.9, -8.9, -8.5, -8.3, -8.0, and -8.0 kcal/mol, respectively. All compounds have the potential for development into drugs for NSCLC treatment. Further in vitro and in vivo investigations are needed to bring these compounds to the clinical setting.

Keywords: *Camellia sinensis*, Catechin Derivatives, Epidermal Growth Factor Receptor, Molecular Docking, Non-Small Cell Lung Carcinoma

1. Introduction

Cancer is one of the biggest health problems with lung cancer as the leading cause of the ever-increasing number of new cases and deaths throughout the world.[1] In 2020, WHO reported that the most frequently diagnosed cancer was lung cancer with 2.21 million cases.[2] In all cancer related deaths, lung cancer is the top in the number of mortality cases with 28% in men and 26% in women.[3] According to the histological type, lung cancer can be classified into two types which are small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC).[4] NSCLC accounts for 85% of all lung cancer cases and five year survival rate of this disease ranges between 10-15%, which is one of the lowest rates among cancers.[5]

In the previous epidemiologic study of epidermal growth factor receptor (EFGR) mutation, NSCLC was reported to be associated with mutation in EGFR genes in about 20% of western patients and 51.4% of Asian patients.[6] EGFR is a kind of tyrosine kinase receptor located at the cell surface that belongs to HER receptor family.[7] EGFR involved in cell signaling pathways that control cell division and survival by promoting cell proliferation and opposing apoptosis. On some types of cancer, mutation in EGFR genes can cause EGFR proteins to be made in higher levels than normal that cause cancer cells to divide rapidly.[8] Based on the fact that EGFR mutation leads to NSCLC, research has shown that targeting EGFR was currently considered as the most suitable way to treat it. However,

75-80% cases cannot respond to EGFR tyrosine kinase inhibitors (TKI) such as gefitinib due to drug resistance in some NCLC patients.[9] Therefore, the development of novel and safe treatment regimens for treating NSCLC patients is needed.

In the last few decades, research on the use of herbs as an alternative treatment with minimal side effects has been developed and Indonesia has great opportunities for herbal medicinal research due to abundant natural resources.[10,11] Previous researches had identified the role of catechins in *Camellia sinensis* (green tea) leaves as antioxidant, anticancer, anti-inflammatory, antimicrobial, and antiviral.[12] In vitro and in vivo studies have determined that theaflavins (one of the catechin derivatives) inhibit various cancer cells, such as ovarian cancer, colorectal cancer, prostate cancer, leukemia, and breast cancer.[13] Gao et al. reported that lower concentrations of theaflavin gallate and theaflavin digallate had inhibitory effects on OVCAR-3 and A2780/CP70 ovarian cancer cells.[14] In addition, green tea leaves also contain other types of catechins, namely epigallocatechin gallate (EGCG) that also reported induced apoptosis in gastric cell lines by downregulating survivin expression.[15]

This study aimed to identify the potential compounds derived from green tea leaves as therapeutic agents to treat NSCLC. Hence, it is necessary to conduct in silico research using molecular docking to predict potential green tea leaves compounds in EGFR as receptor target.

2. Method

2.1 Prediction of Drug-Likeness and ADMET Properties

Drug-likeness prediction was performed using SwissADME, a free online website tool (http://www.swissadme.ch/).[16] Meanwhile, to assessed ADMET properties of the compounds, we used admetSAR, a free online website tool (http://lmmd.ecust.edu.cn/admetsar2).[17]

2.2 Selection of Protein Target and Ligands

We used the term ligand as a compound in green tea or a comparative drug. A total of 12 compounds (ligands) were selected and gefitinib was used as a comparative drug. We selected the ligands through online screening based on previous literatures. A compound that has been proven to be potential in medicinal effect was selected. The structure of ligands were downloaded from Pubchem database(https://pubchem.ncbi.nlm.nih.gov/) whereas EGFR (PDB ID: 3G5Z) as protein targets were downloaded from Protein Data Bank (http://www.rcsb.org).

2.3 Preparation of protein target and ligands

We used Autodock and Discovery Studio software to perform the preparation. The preparation of protein target was carried out by removing water molecules, adding polar hydrogen atoms, and removing a natural ligand structure. After the preparation, we saved the file in pdbqt format. Meanwhile, the preparation of ligands were performed by creating all bonds being all rotatable then saved the file in pdbqt format.[18]

2.4 In-Silico molecular docking

We executed docking using Autodock Vina software. A protein target site was set with the help of a grid box parameters shown in **Table 1**. The best binding affinities (more negative value) was selected from a set of nine conformation poses after running docking. A compound showing the best hits was selected to be visualized its molecular interaction.

2.5 Visualization analysis

We performed visualization analysis to assess the binding sites of the ligand and observed chemical bonds formed between ligands and

protein target. The visualization analysis was carried out using Discovery Studio software and depicted in 3D and 2D.

Table 1. Coordinate value and grid box size

		EGFR
Center (Å)		
	x	-58,421
	y	-7,987
	z	-24.573
Box dimension (Å)		
	x	60
	y	60
	z	60

Table 2. Lipinski rule of five results

Compounds	MW <500 (g/mol)	H-donor	H-acceptor	LogP	Molar Refractivity
Caffein	194.19	0	3	-0.50	52.04
Catechin Gallate	442.37	7	10	1.04	110.04
Catechin	290.27	5	6	0.98	74.33
Epicatechin Gallate	442.37	7	10	1.04	112.06
Epicatechin	290.27	5	6	0.98	110.04
Epigallocatechin-3-gallate	458.37	8	11	0.57	74.33
Kaempferol	302.36	5	6	-0.90	74.77
Myricetin	318.24	6	8	1.06	80.06
Quercetin	318.36	6	7	-1.80	75.93
Theaflavin gallate	716.60	11	16	1.67	215.39
Theaflavin digallate	868.70	13	20	1.42	179.69
Thearubigin	406.34	6	10	0.67	97.70

3. Results

3.1 Drug-Likeness and ADMET Properties

Drug-likeness and ADMET properties were assesed using lipinski rules and admetSAR to determine the toxicity of the compounds. Based on Lipinski's Rule of Five that showed in **Table 2**, most of the compounds used in this study have complied with all the Lipinski Five of Rule so that most of the compounds used in this study were considered drug-like compounds. In a meantime, based on admetSAR in **Table 3**, all compounds in this study had a "-" value on carcinogenicity. It indicates that all compounds in this study were non-carcinogenic. Meanwhile, the ames-mutagenesis showed that most of the compounds in this study had a "-" value means non-mutagenic. In assessing acute oral toxicity, almost all compounds in this study had LD_{50} > 500 mg/kg so all compounds were categorized in III and IV, which means non-toxic.

3.2 In-Silico molecular docking

The docking results are presented in **Table 4.** Eight compounds such as theaflavin gallate, theaflavin digallate, epicatechin gallate, EGCG, catechin gallate, thearubigin, kaempferol, and quercetin had the higher binding energy of -10.6, -9.8, -8.9, -8.9, -8.5, -8.3, -8.0, and -8.0 kcal/mol, respectively than the binding energy of gefitinib. Moreover, the binding energy of myricetin and catechin were same to gefitinib which was -7.9 kcal/mol. Meanwhile, only epicatechin and caffein showed lower binding energy than gefitinib which were -7.5 and -5.4 kcal/mol. Top four compounds and gefitinib were selected to visualize their interaction.

Table 3. Toxicity

Compounds	Carcinogenecity	Ames mutagenesis	Acute oral toxicity
Caffein	- (0.9429)	- (0.8800)	II (0.7405)
Catechin	- (0.9286)	+ (0.6300)	IV (0.6433)
Catechin Gallate	- (0.9857)	- (0.5000)	IV (0.3764)
Epigallocatechin-3-gallate	- (0.9857)	- (0.6700)	IV (0.3764)
Epicatechin Gallate	- (0.9857)	- (0.5000)	IV (0.3764)
Epicatechin	- (0.9286)	+ (0.6300)	IV (0.6433)
Kaempferol	- (0.9714)	- (0.5100)	III (0.4731)
Myricetin	- (1.000)	+ (0.5300)	II (0.7348)
Quercetin	- (0.9714)	- (0.6400)	III (0.4731)
Theaflavin digallate	- (0.9857)	+ (0.5100)	II (0.3538)
Theaflavin gallate	- (0.9429)	- (0.5000)	IV (0.3423)
Thearubigin	- (0.8900)	- (0.7300)	III (0.5681)

Table 4. Molecular docking results

Compounds	Binding Energy (kcal/mol)
Catechin	-7.9
Myricetin	-7.9
Theaflavin gallate	-10.6
Theaflavin digallate	-9.8
Epicatechin gallate	-8.9
Epigallocatechin-3-gallate	-8.9
Catechin gallate	-8.5
Thearubigin	-8.3
Quercetin	-8.0
Kaempferol	-8.0
Caffein	-5.4
Epicatechin	-7.5

Table 5. The summary of visualization results

Compounds	Amino Acid Residues	Molecular Interaction	
		Hydrophobic Interaction	Hydrogen bond
Theaflavin digallate	Asp800, Gly796(2), Leu844, Arg841(2), Asn842, Val726(2), Lys745(2), Glu762, Thr790, Gly721, Ala722, Cys797, Leu799	7	9
Theaflavin gallate	Leu718(3), Gly721, Ala722, Val726(3), Leu844(2), Cys797	9	2
Epicatechin gallate	Met793, Val726, Thr854, Lys745(2), Arg841, Asn842, Cys797(2), Leu844, Leu718(2)	6	6
EGCG	Met793, Leu718, Leu844, Cys797(2), Asn842, Asp855(3), Lys745(2), Val726, Thr854	7	6
Gefitinib	Leu718, Gly796, Val726(2), Gly721, Ser720, Ala722, Gly724, Ala743(2), Leu844(2)	3	4

3.3 Visualization Analysis

There were many amino acid residues that targeted by four compounds as shown in **Table 5.** The visualization of molecular docking results are shown in 3D form in **Figure 1** and 2D form in **Figure 2.** Most of the compounds targeted amino acid residues of Cys797, Leu844, Val726, Leu718, Asn842, Lys745, Arg841, and Gly721. All compounds had two type of interactions which were hydrophobic and hydrogen bond interactions.

4. Discussion

Overexpression of EGFR leads to develop NSCLC. The overexpression occur in 89% of NSCLC patients.[19,20] Gefitinib remains as a first line cancer therapy.[21] Unfortunately, administrating gefitinib sometimes lead to the resistance in 45-60% of NSCLC tumors through the mutation in EGFR. This mutation promotes cellular growth and proliferation.[22] Previous study stated that the way to stop EGFR activity effectively is to block its interactions with ATP, then this receptor cannot functionate normally in activating several pathways. [23]

In this study, it was proven that many compounds exhibited strong binding affinities to EGFR because those compounds had more negative value than gefitinib. Caffein was the only compound that posed the least binding energy. This docking results suggest that the compounds of green tea leaves can interact with EGFR. Top three compounds exhibited the highest binding affinities (lowest binding energies) were selected to analyze its molecular interactions. In this study, theaflavin gallate, theaflavin digallate, epicatechin gallate, and EGCG bound in the active sites (binding pocket) of EGFR.

Previous study showed that amino acid residues located in the active sites were Cys797, Arg841, Asn842, Leu792, and Thr854.[24] Rasyid et al. also reported that the
binding sites of EGFR were amino acid residues of Ala743, Phe795, Met793, Pro794, Leu792, Gln791, Thr790, Asp855, Arg841, Thr854, Leu844, Arg841, Thr854, Asp800, Cys797, Gly796, and Leu718.[25] Cys797 and Leu844 were found in all compound interactions. Leu844 was also found in all interactions including a

comparative drug, gefitinib. This indicates that the compounds interacted in the active sites of EGFR. A docking study also suggested that amino acid residues in the binding pocket of the receptor who interacted with the ligands via hydrophobic interactions were Leu844, Met790, Phe723, Leu718, Leu792, Cys797, and Ala743.[26] In line with previous study, our findings showed that Cys797 in epicatechin gallate, theaflavin gallate, and EGCG interactions were found to have hydrophobic interactions. Besides, cys797 interacted via hydrogen bond in theaflavin digallate. This study also found that amino acid residue of Lys745 was seen to interact with ligand via hydrogen bond interactions.[26] In line with our findings suggested that Lys745 binds to theaflavin digallate and epicatechin gallate via hydrogen bond interactions.

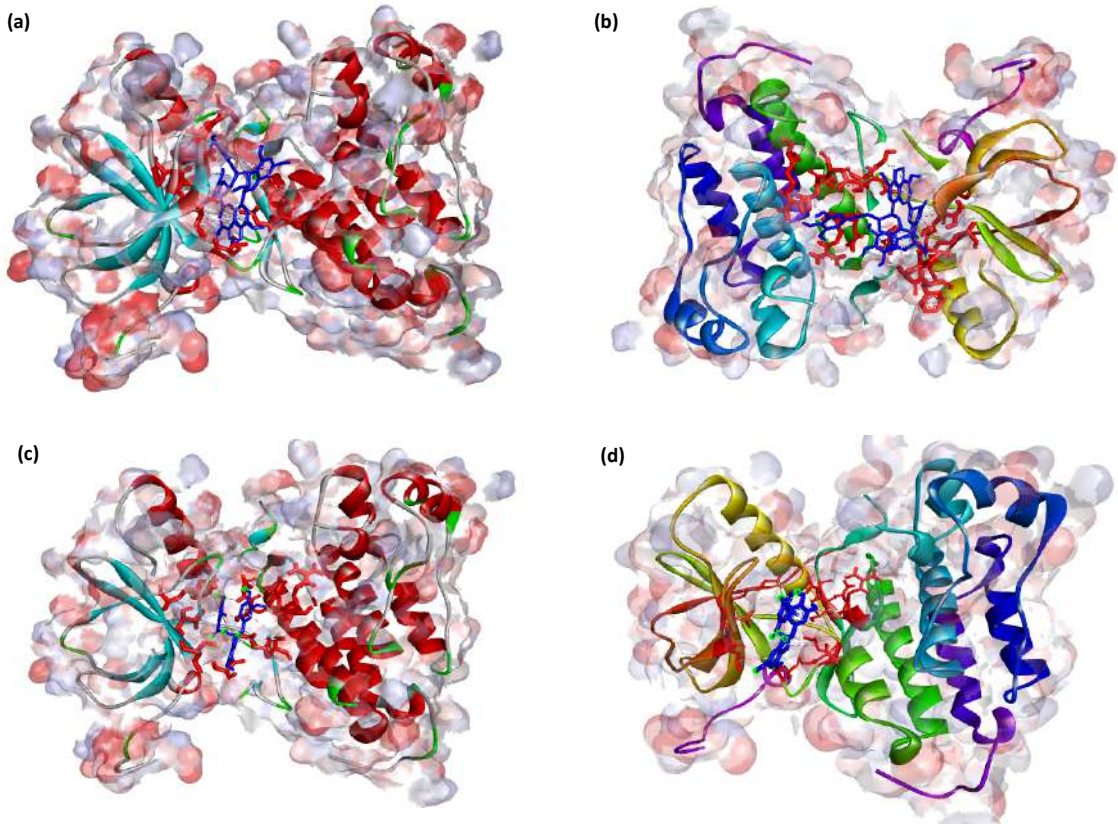

Figure 1. 3D structures of interaction between EGFR and (a) theaflavin gallate; (b) theaflavin digallate; (c) epicatechin gallate; and (d) EGCG.

The most highly potent and selective analogue inhibitor of EGFR was found to bind with amino acid recidues of Cys797, Leu844, and Val726.[27] In addition, sea urchin (*Arbacia lixula*) peptide was found to interact with residues Cys797, Asn842, Lys728, Met793, Arg841, Lys745, and Gly721.[28] Sea urchin extract was found to have anticancer activity in which it could inhibit the cycle cell, migration, and proliferation of breast cancer cell. [29,30,31]

Lipinski's rule of five describes a relationship between pharmacokinetics and physicochemical parameters.[32] The Lipinski rule of five can help to distinguish between drug-like compounds and non-drug-like compounds. The rules consist of molecular weight less than 500 Dalton,

number of H-bond acceptors less than 10, number of H-bond donors less than 5, LogP less than 5, and molar refractivity should be between 40-130.[33] In this study, most of the compounds were considered drug-like compounds.

Toxicity assessment in this study used admetSAR. We used three indicators in assessing toxicity, namely carcinogenecity, ames mutagenesis, and acute oral toxicity. Carcinogenicity test shows the results of whether a compound is carcinogenic or not. In this study, all compounds showed negative results so that all compounds were non-carcinogenic. Ames toxicity test is helpful to determine whether a compound is mutagenic or not. Almost all of the compounds showed negative results, which means non-mutagenic. Acute oral toxicity has four categories to state

whether the compound is oral toxic or not. Category I ($LD_{50} \leq 50$ mg/kg) and category II (50 mg/kg < $LD_{50} \leq 500$ mg/kg) considered as toxic while category III (500 mg/kg < $LD_{50} \leq 5000$ mg/kg) and category IV ($LD_{50} > 5000$ mg/kg) considered as non-toxic. Most of the compounds had category III and IV which was non-toxic.[17,34]

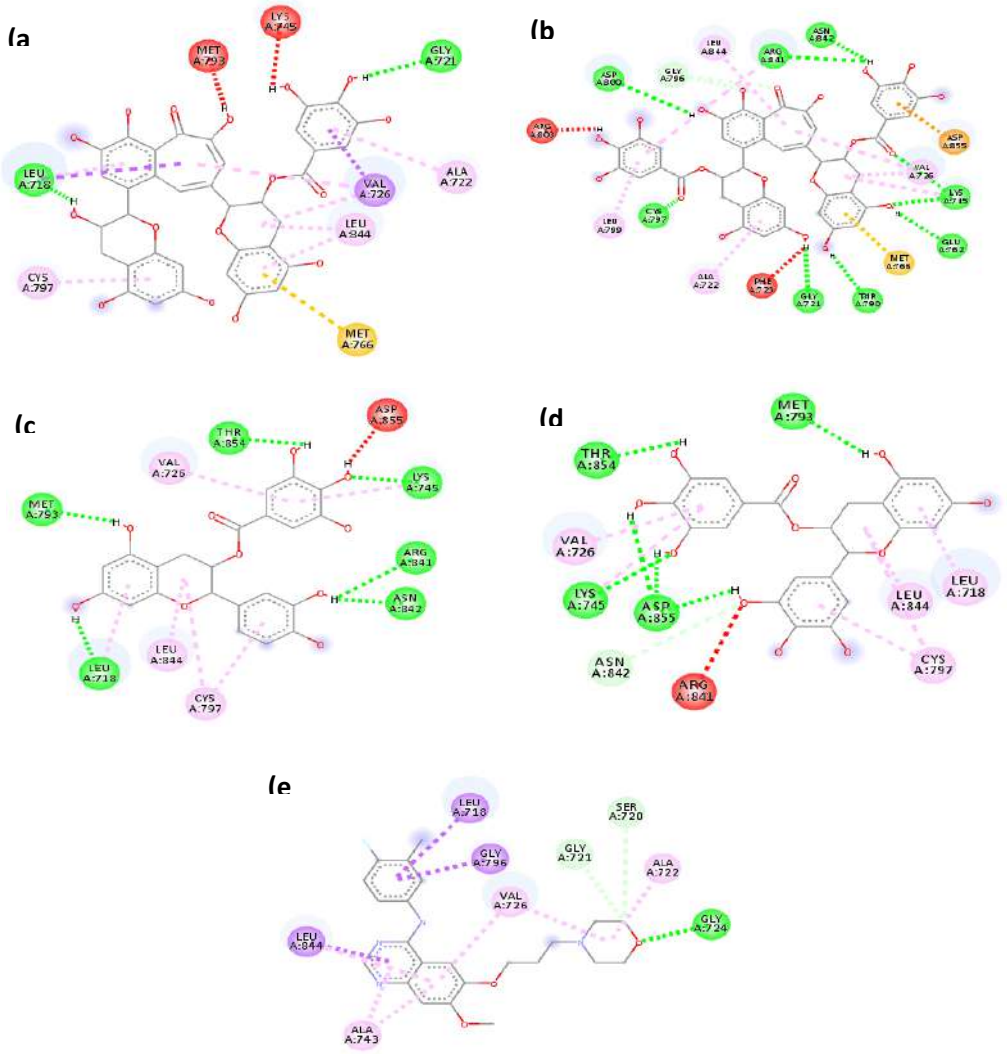

Figure 2. 2D structures of interaction between EGFR and (a) theaflavin gallate; (b) theaflavin digallate; (c) epicatechin gallate; (d) EGCG; and (e) gefitinib.

In vivo study conducted by Hsu et al. showed that given green tea extract as much 625, 1.250, and 2.500 mg/kg/day intragastric for 28 days to mice did not affect the mortality rate caused by toxicity of green tea. In vitro study conducted by Li et al. showed that *Camellia sinensis* flower extract was tested for ames mutagenesis using strains of S. Typhimurium consisting of TA97, TA98, TA100, TA102, indicating that green tea flower extract was not mutagenic. Thus, the results of the toxicity analysis in this study were in line with previous studies where green tea did not produce a toxic effect significantly that tested in vivo and in vitro.[35,36]

5. Conclusion

Twelve green tea leaves compounds were selected based on previous study. Docking results showed that all compounds had affinity to EGFR with theaflavin gallate, theaflavin digallate, epicatechin gallate, and EGCG had the strongest binding energy compared to gefitinib and other compounds. All compounds are safe and have the potential for development into drugs for NSCLC treatment. Further in vitro and in vivo investigations are needed to bring these compounds to the clinical setting.

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